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VASCULAR-PREFERRED PROMOTERS

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VASCULAR-PREFERRED PROMOTERS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 60/428,287, filed November 22, 2002.

FIELD OF INVENTION

[0002] The present invention relates to the regulation of polynucleotide transcription and/or expression. In particular, this invention relates to polynucleotide regulatory sequences isolated from *Eucalyptus grandis* and *Pinus radiata* that confer vascular-preferred transcription of polynucleotides in plant cells. Constructs and methods for using the inventive regulatory sequences for modifying transcription of endogenous and/or heterologous polynucleotides also are included in the invention.

BACKGROUND OF THE INVENTION

[0003] A major goal in the forestry and paper industries is the control of lignin content in plants. Lignin is a complex polymer of cinnamyl alcohols that is responsible for wood's mechanical strength, coloration, and resistance to rot. Tree species synthesize large quantities of lignin, with lignin constituting between 20% to 30% of the dry weight of wood. In addition to providing rigidity, lignin aids water transport by rendering cell walls hydrophobic and water-impermeable. It follows that increasing the lignin concentration in trees can prove beneficial for certain applications, such as providing trees with improved disease resistance or increased strength for use in construction. Lignin is also useful as a fuel, and lignin together with increased cellulose content is desirable in wood or other biomass used as fuel, such as wood for charcoal production, corn stover, and trees such as willow and fast growing aspen hybrids used for biofuels.

[0004] Conversely, the high concentration of lignin in trees presents a significant problem for the paper industry, which must expend considerable resources to separate lignin from cellulose fiber. In the U.S. alone, about 20 million tons of lignin are removed from wood per year. Further, the content of lignin is largely responsible for the digestibility, or lack thereof, of forage crops, with small increases in plant lignin content resulting in relatively high decreases in digestibility. For example, crops with reduced lignin content provide more efficient forage for cattle, with the yield of milk and meat being higher relative to the amount of forage crop consumed. Lignin content increases during plant growth, so that farmers must choose between harvesting crops early to obtain a lower yield of more digestible crops or later to obtain a higher yield of less digestible material.

[0005] For these reasons, the control of lignin content or composition through genetic modification of plants is desirable. Considerable effort has been made to this end to identify and characterize the genes responsible for lignin biosynthesis and to determine sequences that regulate their expression. Polynucleotides encoding many of the enzymes involved in lignin biosynthesis have been cloned, including cinnamyl alcohol dehydrogenase (CAD), cinnamate 4-hydroxylase (C4H), coumarate 3-hydroxylase (C3H), phenolase (PNL), O-methyl transferase (OMT), cinnamoyl-CoA reductase (CCR), phenylalanine ammonia-lyase (PAL), 4-coumarate:CoA ligase (4CL) and peroxidase (POX) from pine. U.S. Pat. No. 6,204,434.

[0006] Manipulation of the expression of these genes has been used to modify lignin content. Such experiments include altering the number of copies of genes encoding CAD, coniferin β-glucosidase (CBG), and caffeic acid 3-O-methyltransferase (COMT). U.S. Pat. No. 5,451,514, WO 94/23044, and Dharmawardhana *et al.*, *Plant Mol. Biol.* **40**: 365-72 (1999). Furthermore, antisense expression of sequences encoding CAD in poplar, *N. tabacum*, and pine leads to the production of lignin having a modified composition. Grand *et al.*, *Planta (Berl.)* **163**: 232-37 (1985), Yahiaoui *et al.*, *Phytochemistry* **49**: 295-306 (1998), and Baucher *et al.*, *Plant Physiol.* **112**: 1479 (1996), respectively.

[0007] Another major goal of the forest products, paper, plant biomass and forage industries is to increase the size of the stem, to manipulate cellulose content in the stem, or to manipulate characteristics of the cell wall in order to facilitate the recovery of cellulose from the stem. For example, cellulose is recovered from the xylem fibers in pulp production, and the number of xylem fibers and vessel elements, thickness of the cell walls, diameter of the cell lumens, length of the fibers, cellulose microfibril angle and other characteristics of these xylem cells determine the quality and quantity of cellulose recovered. Manipulation of genes involved in cellulose biosynthesis has been useful to increase the total biomass of plants and the yield of cellulose from the plants, while antisense expression of such genes has demonstrated effects on cell wall development. Shani Z., Shpigel, E., Roiz, L., Goren, R., Vinocur, B., Tzfira, T., Altman, A., and Shoseyov O. Cellulose binding domain increases cellulose synthase activity in Acetobacter xylinum, and biomass of transgenic plants. In: A. Altman, M. Ziv, S. Izhar, eds., Plant Biotechnology and In Vitro Biology in the 21st Century, pp. 213-218 Kluwer Academic Publishers. (1999). Modification of polysaccharides and plant cell wall by endo-1,4-β-glucanase and cellulose-binding domains has been described. Levy, I., Shani, Z. and Shoseyov O.Biomol Eng. **19**: 17-30 (2002). Accordingly, the polynucleotides of the instant invention can be used to express nucleotide sequences in vascular tissue to modify cellulose biosysnthesis thereby affecting plant growth and biomass.

[0008] Genetic regulation of biochemical pathways preferably is conducted in narrowly restricted tissue types to avoid global, detrimental effects to the modified plants. For example, when the content or composition of lignin is affected by expression of a particular gene product, it may be desirable to limit the expression of the gene product to certain segments of the plant or to certain developmental stages, to avoid decreasing the plant's disease resistance. A heterologous gene may be expressed in a selected tissue by operably linking it to a tissue-preferred promoter. Suitable tissue-preferred promoters include the bean *grp1.8* promoter, which is specifically active in protoxylem tracheary elements of vascular tissue. Keller *et al.*, *EMBO J.* **8**: 1309 (1989). These promoters also include the eucalyptus *CAD* promoter, which is preferentially expressed in lignifying zones. Feuillet *et al.*, *Plant*

Mol. Biol. **27**: 651 (1995). Such tissue-preferred promoters have been used to regulate gene expression of antisense molecules in specific tissues. Van der Meer *et al.*, *Plant Cell* **4**: 253 (1992), Salehuzzaman *et al.*, *Plant Mol. Biol.* **23**: 947 (1993), and Matsuda *et al.*, *Plant Cell Physiol.* **37**: 215 (1996).

[0009] Because tissue-preferred promoters may be less active in a heterologous environment, they do not always express genes to the same levels achieved with constitutive promoters. Yahiaoui *et al.*, *Phytochemistry* **49**: 295-306 (1998). Further, the developmental window during which these promoters are active, or the spatial distribution of their activity, may limit their usefulness. Thus, there is a continuing need in the art to define additional tissue-preferred promoters, especially vascular-preferred promoters, that have desirable spatial and temporal patterns of expression. Reviewed by Grima-Pettenati *et al.*, *Plant Science* **145**: 51-65 (1999).

SUMMARY OF THE INVENTION

[0010] The present invention relates to isolated polynucleotide regulatory sequences that confer vascular-preferred gene expression. The polynucleotides of the present invention can be used for controlling the lignin content, cellulose content, size or cell wall development of a plant. The polynucleotides of the present invention may also be used for regulating biosynthesis of lignin, cellulose, and plant cell walls.

[0011] In one aspect, the present invention provides an isolated nucleic molecule comprising a polynucleotide that is capable of conferring vascular-preferred polynucleotide transcription. In one embodiment, the isolated nucleic molecule comprises a polynucleotide selected from any one of SEQ ID NO: 1-85 and functional variants thereof. In another embodiment, the functional variant has a sequence identity that is greater than or equal to 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, or 60% in sequence to any one of SEQ ID NO: 1-85.

[0012] In another aspect, the present invention provides an isolated polynucleotide having a sequence selected from (a) sequences complementary to any one of SEQ ID NO: 1-85 and functional variants thereof, (b) sequences that are reverse complements to any of the sequences in SEQ ID NO: 1-85; and (c) sequences comprising at least 20 contiguous bases, which hybridizes to any of the polynucleotides of (a) or (b). In one embodiment, the present invention contemplates wherein said polynucleotide confers xylem-preferred gene expression in a plant cell. In another embodiment, the present invention provides a polynucleotide capable of upregulating or downregulating the expression of an operably-linked gene in a plant cell.

[0013] In another aspect, the present invention provides a plant cell comprising (a) at least one polynucleotide sequence that has the sequence of any one of SEQ ID NO: 1-85, and (b) a desired gene, wherein said polynucleotide and said desired gene are operably linked. In one embodiment, the desired gene encodes a polypeptide or protein. In another embodiment, the protein is an enzyme involved in the biosynthesis of cell walls. In a further embodiment, the protein is an enzyme involved in lignin biosynthesis. In another embodiment, the desired gene produces an RNA transcript. In yet another embodiment, the RNA transcript has an antisense sequence of a gene that is endogenous to a plant cell. In an further embodiment, the RNA transcript induces RNA interference of a gene that is normally expressed in a plant cell.

[0014] In another aspect, the present invention provides a plant comprising a plant cell having (a) at least one polynucleotide sequence that has the sequence of any one of SEQ ID NO: 1-85, and (b) a desired gene, wherein said polynucleotide and said desired gene are operably linked. In one embodiment, the plant is selected from angiosperms and gymnosperms.

[0015] In another embodiment, the present invention contemplates a method for regulating the lignin content of a plant, comprising cultivating the plant comprising a plant cell comprising a DNA construct that comprises (a) at least one polynucleotide

sequence that has the sequence of any one of SEQ ID NO: 1-85, and (b) a desired gene, wherein said polynucleotide and said desired gene are operably linked.

[0016] In another aspect, the present invention contemplates a method for regulating cell wall development in a plant, comprising cultivating the plant comprising a plant cell comprising a DNA construct that comprises (a) at least one polynucleotide sequence that has the sequence of any one of SEQ ID NO: 1-85, and (b) a desired gene, wherein said polynucleotide and said desired gene are operably linked.

[0017] In another aspect, the present invention provides a transgenic plant comprising a polynucleotide sequence selected from any one of SEQ ID NO: 1-85 and functional variants thereof.

[0018] In one aspect, the present invention provides a method for obtaining wood, comprising (a) introducing into a plant cell of a woody plant a DNA construct comprising (i) a promoter having the sequence of any one of SEQ ID NOs: 1 to 85 or functional variants thereof and (ii) and a desired nucleic acid, wherein said promoter regulates the expression of said desired nucleic acid; (b) culturing said transformed plant cell under conditions that promote growth of a plant; and (c) obtaining wood from said plant. In one embodiment, the woody plant is selected from a species of *Eucalyptus* or *Pinus*.

[0019] The present invention also contemplates an isolated polynucleotide sequence selected from the group consisting of SEQ ID NO: 1-85, and polynuclucleotides having at least 60% sequence identity to any of these sequences. The invention also contemplates a polynucleotide having at least 65% sequence identity to any one of SEQ ID NO: 1-85. The invention further contemplates a polynucleotide having at least 70% sequence identity to any one of SEQ ID NO: 1-85. The present invention provides an isolated polynucleotide sequence having at least 75% sequence identity to any of SEQ ID NO: 1-85. The invention contemplates a polynucleotide having at least 80% sequence identity to any of SEQ ID NO: 1-85. Also provided is an isolated polynucleotide having 85% sequence identity to any one of SEQ ID NO: 1-85. The present invention contemplates sequences having at least 90% sequence identity to

any one of SEQ ID NO: 1-85. Sequences having at least 95% sequence identity with any of SEQ ID NO: 1-85 are provided by the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] FIG. 1 depicts a Zinnia TE-based assay demonstrating three promoters having a similar expression profiles at Day 3 of culture.

[0021] FIGS. 2 depicts a Zinnia TE-based assay demonstrating two promoters having a similar expression profile at Day 3 of culture.

[0022] FIG. 3 depicts a Zinnia TE-based assay demonstrating two promoters having a similar expression profile at Day 3 of culture.

DETAILED DESCRIPTION OF THE EMBODIMENTS

[0023] The present invention relates to an isolated nucleic molecule comprising a polynucleotide having a sequence at least 95% identical to a sequence selected from the group consisting of any of the polynucleotide sequences set forth in Table 6. The invention also provides functional fragments of the polynucleotide sequences disclosed in Table 6. The invention further provides complementary nucleic acids, or fragments thereof, to any of the polynucleotide sequences listed in Table 6, as well as a nucleic acid, comprising at least 15 contiguous bases, which hybridizes to any of the polynucleotide sequences disclosed in Table 6

[0024] The present invention relates to an isolated nucleic molecule comprising a polynucleotide having a sequence at least 95% identical to a sequence selected from the group consisting of any of the polynucleotide sequences set forth in Table 6. The invention also provides functional fragments of the polynucleotide sequences disclosed in Table 6. The invention further provides complementary nucleic acids, or fragments thereof, to any of the polynucleotide sequences listed in Table 6, as well as a nucleic acid, comprising at least 15 contiguous bases, which hybridizes to any of the polynucleotide sequences disclosed in Table 6.

[0025] The nucleotide sequences of the nucleic acids of the invention are provided in a sequence listing. What is intended by "SEQ ID NO: (2N+1)" are all the odd numbered sequences in the sequence listing, e.g., 1, 3, 5, 7 etc. What is intended by "SEQ ID NO: 2N" are the even numbered sequences. The difference between an odd numbered sequence, and the immediately proceeding even numbered sequence (e.g, SEQ ID NO: 1 and SEQ ID NO:2) should be that the even numbered sequence has additional bases. The last three bases of the even numbered sequences are usually ATG and represent the start codon (usually coding for methionine) for translation.

[0026] The present invention uses terms and phrases that are well known to those practicing the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, and nucleic acid chemistry and hybridization described herein are those well known and commonly employed in the art. Standard techniques are used for recombinant nucleic acid methods, polynucleotide synthesis, microbial culture, cell culture, tissue culture, transformation, transfection, transduction, analytical chemistry, organic synthetic chemistry, chemical syntheses, chemical analysis, and pharmaceutical formulation and delivery. Generally, enzymatic reactions and purification and/or isolation steps are performed according to the manufacturers' specifications. The techniques and procedures are generally performed according to conventional methodology. *See, e.g.,* Sambrook & Russel, MOLECULAR CLONING: A LABORATORY MANUAL, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001.

[0027] ***Agrobacterium***: as is well known in the field, *Agrobacteria* that are used for transforming plant cells are disarmed and virulent derivatives of, usually, *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* that contain a vector. The vector typically contains a desired polynucleotide that is located between the borders of a T-DNA.

[0028] ***Angiosperm**:* vascular plants having seeds enclosed in an ovary. Angiosperms are seed plants that produce flowers that bear fruits. Angiosperms are divided into dicotyledonous and monocotyledonous plants.

[0029] ***Desired Polynucleotide**:* a desired polynucleotide of the present invention is a genetic element, such as a promoter, enhancer, or terminator, or gene or polynucleotide that is to be transcribed and/or translated in a transformed cell that comprises the desired polynucleotide in its genome. If the desired polynucleotide comprises a sequence encoding a protein product, the coding region may be operably linked to regulatory elements, such as to a promoter and a terminator, that bring about expression of an associated messenger RNA transcript and/or a protein product encoded by the desired polynucleotide. Thus, a "desired polynucleotide" may comprise a gene that is operably linked in the 5'- to 3'- orientation, a promoter, a gene that encodes a protein, and a terminator. Alternatively, the desired polynucleotide may comprise a gene or fragment thereof in an "antisense" orientation, the transcription of which produces nucleic acids that may form secondary structures that affect expression of an endogenous gene in the plant cell. A desired polynucleotide may also yield a double-stranded RNA product upon transcription that initiates RNA interference of a gene to which the desired polynucleotide is associated. A desired polynucleotide of the present invention may be positioned within a T-DNA, such that the left and right T-DNA border sequences flank or are on either side of the desired polynucleotide. The present invention envisions the stable integration of one or more desired polynucleotides into the genome of at least one plant cell. A desired polynucleotide may be mutated or may be a variant of its wild-type sequence. It is understood that all or part of the desired polynucleotide can be integrated into the genome of a plant. It also is understood that the term "desired polynucleotide" encompasses one or more of such polynucleotides. Thus, a T-DNA of the present invention may comprise one, two, three, four, five, six, seven, eight, nine, ten, or more desired polynucleotides.

[0030] ***Dicotyledonous plant (dicot)**:* a flowering plant whose embryos have two seed halves or cotyledons, branching leaf veins, and flower parts in multiples of four

or five. Examples of dicots include but are not limited to, *Eucalyptus, Populus, Liquidamber, Acacia,* teak, mahogany, cotton, tobacco, *Arabidopsis*, tomato, potato sugar beet, broccoli, cassava, sweet potato, pepper, poinsettia, bean, alfalfa, soybean, carrot, strawberry, lettuce, oak, maple, walnut, rose, mint, squash, daisy, geranium, avocado, and cactus.

[0031] Endogenous refers to a gene that is native to a plant genome.

[0032] **Fiber composition**: as used herein, fiber composition refers to a trait that can be modified to change the structure, appearance, or use of fiber. Traits that determine fiber composition include but are not limited to fiber length, coarseness, strength, color, cross-sectional width, and fiber density. For example, it is known that fiber length imparts strength, whereas fiber coarseness is a determinant of texture and flexibility.

[0033] **Foreign**: "foreign," with respect to a nucleic acid, means that that nucleic acid is derived from non-plant organisms, or derived from a plant that is not the same species as the plant to be transformed or is not derived from a plant that is not interfertile with the plant to be transformed, or does not belong to the species of the target plant. According to the present invention, foreign DNA or RNA may include nucleic acids that are naturally occurring in the genetic makeup of fungi, bacteria, viruses, mammals, fish or birds, but are not naturally occurring in the plant that is to be transformed. Thus, a foreign nucleic acid is one that encodes, for instance, a polypeptide that is not naturally produced by the transformed plant. A foreign nucleic acid does not have to encode a protein product.

[0034] **Gene**: A gene is a segment of a DNA molecule that contains all the information required for synthesis of a product, polypeptide chain or RNA molecule, and includes both coding and non-coding sequences.

[0035] **Genetic element**: a "genetic element" is any discreet nucleotide sequence including, but not limited to, a promoter, a gene, a terminator, an intron, an enhancer,

a spacer, a 5'-untranslated region, a 3'-untranslated region, or a recombinase recognition site.

[0036] Genetic modification: stable introduction of DNA into the genome of certain organisms by applying methods in molecular and cell biology.

[0037] Gymnosperm: as used herein, refers to a seed plant that bears seed without ovaries. Examples of gymnosperms include conifers, cycads, ginkgos, and ephedras.

[0038] Introduction: as used herein, refers to the insertion of a nucleic acid sequence into a cell, by methods including infection, transfection, transformation or transduction.

[0039] Juvenility: describes a physiological difference between a young tree and a mature tree. In the present invention, juvenility refers to differences in microfibril angle, wood density, cellulose yield, regenerability, and reproductive ability between a young tree and a mature tree. For example, it has been shown that as a woody plant tissue matures, the tissue loses its ability to regenerate.

[0040] Lignin: as used herein, refers to a polymeric composition composed of phenylpropanoid units, including polymerized derivatives of monolignols coniferyl, coumaryl, and sinapyl alcohol. Lignin quality refers to the ability of a lignin composition to impart strength to cell wall matrices, assist in the transport of water, and/or impede degradation of cell wall polysaccharides. Lignin composition or lignin structure may be changed by altering the relative amounts of each of monolignols or by altering the type of lignin. For example, guaiacyl lignins (derived from ferulic acid) are prominent in softwood species, whereas guaiacyl-syringyl lignins (derived from ferulic acid and sinapic acid) are characteristic of hardwood species. The degradation of lignin from softwoods, such as pine, requires substantially more alkali and longer incubations, compared with the removal of lignin from hardwoods. Lignin composition may be regulated by either up-regulation or down-regulation of enzymes involved lignin biosynthesis. For example, key lignin biosynthsesis enzymes include,

but are not limited to, 4-coumaric acid: coenzyme A ligase (4CL), Cinnamyl Alcohol dehydrogenase (CAD), and Sinapyl Alcohol Dehydrogenase (SAD).

[0041] Monocotyledonous plant (monocot): a flowering plant having embryos with one cotyledon or seed leaf, parallel leaf veins, and flower parts in multiples of three. Examples of monocots include, but are not limited to turfgrass, maize, rice, oat, wheat, barley, sorghum, orchid, iris, lily, onion, and palm. Examples of turfgrass include, but are not limited to *Agrostis spp.* (bentgrass species including colonial bentgrass and creeping bentgrasses), *Poa pratensis* (kentucky bluegrass), *Lolium spp.* (ryegrass species including annual ryegrass and perennial ryegrass), *Festuca arundinacea* (tall fescue) *Festuca rubra commutata* (fine fescue), *Cynodon dactylon* (common bermudagrass varieties including Tifgreen, Tifway II, and Santa Ana, as well as hybrids thereof); *Pennisetum clandestinum* (kikuyugrass), *Stenotaphrum secundatum* (st. augustinegrass), *Zoysia japonica* (zoysiagrass), and *Dichondra micrantha*.

[0042] Operably linked: combining two or more molecules in such a fashion that in combination they function properly in a plant cell. For instance, a promoter is operably linked to a structural gene when the promoter controls transcription of the structural gene.

[0043] Phenotype: phenotype is a distinguishing feature or characteristic of a plant, which may be altered according to the present invention by integrating one or more "desired polynucleotides" and/or screenable/selectable markers into the genome of at least one plant cell of a transformed plant. The "desired polynucleotide(s)" and/or markers may confer a change in the phenotype of a tranformed plant by modifying any one of a number of genetic, molecular, biochemical, physiological, morphological, or agronomic characteristics or properties of the transformed plant cell or plant as a whole. Thus, expression of one or more, stably integrated desired polynucleotide(s) in a plant genome may yield a phenotype selected from the group consisting of, for example, increased drought tolerance, enhanced cold and frost tolerance, improved vigor, enhanced color, enhanced health and nutritional characteristics, improved storage,

enhanced yield, enhanced salt tolerance, enhanced heavy metal tolerance, increased disease tolerance, increased insect tolerance, increased water-stress tolerance, enhanced sweetness, improved vigor, improved taste, improved texture, decreased phosphate content, increased germination, increased micronutrient uptake, improved starch composition, improved flower longevity, altered density, altered stem strength or stem stiffness, increased dimensional stability, and altered cellulose or lignin content.

[0044] Plant tissue: a "plant" is any of various photosynthetic, eukaryotic, multicellular organisms of the kingdom *Plantae* characteristically producing embryos, containing chloroplasts, and having cellulose cell walls. A part of a plant, *i.e.*, a "plant tissue" may be transformed according to the methods of the present invention to produce a transgenic plant. Many suitable plant tissues can be transformed according to the present invention and include, but are not limited to, somatic embryos, pollen, leaves, stems, calli, stolons, microtubers, and shoots. Thus, the present invention envisions the transformation of angiosperm and gymnosperm plants such as turfgrass, wheat, maize, rice, barley, oat, sugar beet, potato, tomato, tobacco, alfalfa, lettuce, carrot, strawberry, cassava, sweet potato, geranium, soybean, oak, apple, grape, pine, fir, acacia, eucalyptus, walnut, and palm. According to the present invention "plant tissue" also encompasses plant cells. Plant cells include suspension cultures, callus, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, seeds and microspores. Plant tissues may be at various stages of maturity and may be grown in liquid or solid culture, or in soil or suitable media in pots, greenhouses or fields. A plant tissue also refers to any clone of such a plant, seed, progeny, propagule whether generated sexually or asexually, and descendents of any of these, such as cuttings or seed. Of particular interest are conifers such as pine, fir and spruce, monocots such as Kentucky bluegrass, creeping bentgrass, maize, and wheat, and dicots such as cotton, tomato, lettuce, *Arabidopsis*, tobacco, apple and geranium.

[0045] Plant transformation and cell culture: broadly refers to the process by which plant cells are genetically modified and transferred to an appropriate plant

culture medium for maintenance, further growth, and/or further development. Such methods are well known to the skilled artisan.

[0046] **Progeny**: a "progeny" of the present invention, such as the progeny of a transgenic plant, is one that is born of, begotten by, or derived from a plant or the transgenic plant. Thus, a "progeny" plant, *i.e.*, an "F1" generation plant is an offspring or a descendant of the transgenic plant produced by the inventive methods. A progeny of a transgenic plant may contain in at least one, some, or all of its cell genomes, the desired polynucleotide that was integrated into a cell of the parent transgenic plant by the methods described herein. Thus, the desired polynucleotide is "transmitted" or "inherited" by the progeny plant. The desired polynucleotide that is so inherited in the progeny plant may reside within a T-DNA construct, which also is inherited by the progeny plant from its parent. The term "progeny" as used herein also may be considered to be the offspring or descendants of a group of plants. A transgenic plant of the instant invention can be asexually reproduced to produce progeny plants.

[0047] **Promoter**: is intended to mean a nucleic acid, preferably DNA, that binds RNA polymerase and/or other transcription regulatory elements. As with any promoter, the promoter sequences of the current present invention will facilitate or control the transcription of DNA or RNA to generate an mRNA molecule from a nucleic acid molecule that is operably linked to the promoter. As stated earlier, the RNA generated may code for a protein or polypeptide or may code for an RNA interfering, or antisense molecule.

[0048] A promoter, as used herein, may also include regulatory elements. Conversely, a regulatory element may also be separate from a promoter. Regulatory elements confer a number of important characteristics upon a promoter region. Some elements bind transcription factors that enhance the rate of transcription of the operably linked nucleic acid. Other elements bind repressors that inhibit transcription activity. The effect of transcription factors on promoter activity may determine

whether the promoter activity is high or low, i.e. whether the promoter is "strong" or "weak."

[0049] A **plant promoter** is a promoter capable of initiating transcription in plant cells, whether or not its origin is a plant cell. Exemplary plant promoters include, but are not limited to, those that are obtained from plants, plant viruses, and bacteria such as *Agrobacterium* or *Rhizobium* which comprise genes expressed in plant cells. Examples of promoters under developmental control include promoters that preferentially initiate transcription in certain tissues, such as xylem, leaves, roots, or seeds. Such promoters are referred to as **tissue preferred promoters**. Promoters which initiate transcription only in certain tissues are referred to as **tissue specific promoters**. A **cell type specific promoter** primarily drives expression in certain cell types in one or more organs, for example, vascular cells in roots or leaves. An **inducible** or **repressible promoter** is a promoter which is under environmental control. Examples of environmental conditions that may effect transcription by inducible promoters include anaerobic conditions or the presence of light. Tissue specific, tissue preferred, cell type specific, and inducible promoters constitute the class of non-constitutive promoters. A **constitutive promoter** is a promoter which is active under most environmental conditions, and in most plant parts.

[0050] **Polynucleotide** is a nucleotide sequence comprising a gene coding sequence or a fragment thereof (comprising at least 15 consecutive nucleotides, at least 30 consecutive nucleotides, or at least 50 consecutive nucleotides), a promoter, an intron, an enhancer region, a polyadenylation site, a translation initiation site, 5' or 3' untranslated regions, a reporter gene, a selectable marker or the like. The polynucleotide may comprise single stranded or double stranded DNA or RNA. The polynucleotide may comprise modified bases or a modified backbone. The polynucleotide may be genomic, an RNA transcript (such as an mRNA) or a processed nucleotide sequence (such as a cDNA). The polynucleotide may comprise a sequence in either sense or antisense orientations.

[0051] An **isolated polynucleotide** is a polynucleotide sequence that is not in its native state, e.g., the polynucleotide is comprised of a nucleotide sequence not found in nature, or the polynucleotide is separated from nucleotide sequences to which it typically is in proximity, or is in proximity to nucleotide sequences with which it typically is not in proximity.

[0052] **Regenerability**: as used herein, refers to the ability of a plant to redifferentiate from a de-differentiated tissue.

[0053] **Seed**: a "seed" may be regarded as a ripened plant ovule containing an embryo, and a propagative part of a plant, as a tuber or spore. Seed may be incubated prior to *Agrobacterium*-mediated transformation, in the dark, for instance, to facilitate germination. Seed also may be sterilized prior to incubation, such as by brief treatment with bleach. The resultant seedling can then be exposed to a desired strain of *Agrobacterium*.

[0054] **Selectable/screenable marker**: a gene that, if expressed in plants or plant tissues, makes it possible to distinguish them from other plants or plant tissues that do not express that gene. Screening procedures may require assays for expression of proteins encoded by the screenable marker gene. Examples of such markers include the beta glucuronidase (*GUS*) gene and the luciferase (*LUX*) gene. Examples of selectable markers include the neomycin phosphotransferase (*NPTII*) gene encoding kanamycin and geneticin resistance, the hygromycin phosphotransferase (*HPT* or *APHIV*) gene encoding resistance to hygromycin, acetolactate synthase (*als*) genes encoding resistance to sulfonylurea-type herbicides, genes (*BAR* and/or *PAT*) coding for resistance to herbicides which act to inhibit the action of glutamine synthase such as phosphinothricin (Liberty or Basta), or other similar genes known in the art.

[0055] **Sequence identity**: as used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences includes reference to the residues in the two sequences which are the same when aligned for maximum correspondence over a specified region.

[0056] As used herein, percentage of sequence identity means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

[0057] **Transcription factor**: Transcription factor refers to a polypeptide sequence that regulates the expression of a gene or genes by either directly binding to one or more nucleotide sequences associated with a gene coding sequence or indirectly affecting the activity of another polypeptide(s) that bind directly to one or more nucleotide sequences associated with a gene coding sequence. A transcription factor may **activate (up-regulate)** or **repress (down-regulate)** expression of a gene or genes. A transcription factor may contain a DNA binding domain, an activation domain, or a domain for protein-protein interactions. In the present invention, a transcription factor is capable of at least one of (1)binding to a nucleic acid sequence or (2) regulating expression of a gene in a plant. Additionally, the inventive polynucleotide sequences and the corresponding polypeptide sequences function as transcription factors in any plant species, including angiosperms and gymnosperms.

[0058] **Transcription and translation terminators**: The expression DNA constructs of the present invention typically have a transcriptional termination region at the opposite end from the transcription initiation regulatory element. The transcriptional termination region may be selected, for stability of the mRNA to enhance expression and/or for the addition of polyadenylation tails added to the gene transcription product.

[0059] Transfer DNA (T-DNA): an *Agrobacterium* T-DNA is a genetic element that is well-known as an element capable of integrating a nucleotide sequence contained within its borders into another genome. In this respect, a T-DNA is flanked, typically, by two "border" sequences. A desired polynucleotide of the present invention and a selectable marker may be positioned between the left border-like sequence and the right border-like sequence of a T-DNA. The desired polynucleotide and selectable marker contained within the T-DNA may be operably linked to a variety of different, plant-specific (*i.e.*, native), or foreign nucleic acids, like promoter and terminator regulatory elements that facilitate its expression, *i.e.*, transcription and/or translation of the DNA sequence encoded by the desired polynucleotide or selectable marker.

[0060] Transformation of plant cells: A process by which a nucleic acid is stably inserted into the genome of a plant cell. Transformation may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of nucleic acid sequences into a prokaryotic or eukaryotic host cell, including *Agrobacterium*-mediated transformation protocols, viral infection, whiskers, electroporation, microinjection, polyethylene glycol-treatment, heat shock, lipofection and particle bombardment.

[0061] Transgenic plant: a transgenic plant of the present invention is one that comprises at least one cell genome in which an exogenous nucleic acid has been stably integrated. According to the present invention, a transgenic plant is a plant that may comprise only one genetically modified cell and cell genome, or it may comprise several or many genetically modified cells, or all of the cells may be genetically modified. A transgenic plant of the present invention may be one in which expression of the desired polynucleotide, *i.e.*, the exogenous nucleic acid, occurs in only certain parts of the plant. Thus, a transgenic plant may contain only genetically modified cells in certain parts of its structure.

[0062] Variant: a "variant," as used herein, is understood to mean a nucleotide sequence that deviates from the reference (i.e., native, standard, or given) nucleotide

sequence of a particular gene. The terms, "isoform," "isotype," and "analog" also refer to "variant" forms of a nucleotide sequence.

[0063] "Variant" may also refer to a "shuffled gene" such as those described in Maxygen-assigned patents. For instance, a variant of the present invention may include variants of sequences and desired polynucleotides that are modified according to the methods and rationale disclosed in U.S. 6,132,970, which is incorporated herein by reference.

[0064] **Wood composition**, as used herein, refers to a trait that can be modified to change the structure, appearance, or use of wood. While not limiting, traits that determine wood composition include cell wall thickness, cell length, cell size, lumen size, cell density, microfibril angle, tensile strength, tear strength, wood color, and length and frequency of cell division.

[0065] **Wood pulp** refers to fiber generated from wood having varying degrees of purification. Wood pulp can be used for producing paper, paper board, and chemical products.

[0066] It is understood that the present invention is not limited to the particular methodology, protocols, vectors, and reagents, etc., described herein, as these may vary. It is also to be understood that the terminology used herein is used for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention. It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a gene" is a reference to one or more genes and includes equivalents thereof known to those skilled in the art and so forth. Indeed, one skilled in the art can use the methods described herein to express any native gene (known presently or subsequently) in plant host systems.

[0067] By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example,

recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include in vitro RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules, according to the present invention, further include such molecules produced synthetically.

[0068] Nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA or RNA may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

[0069] Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 3700 from Applied Biosystems, Inc.), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 95% identical, more typically at least about 96% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence may be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

[0070] Unless otherwise indicated, each "nucleotide sequence" set forth herein is presented as a sequence of deoxyribonucleotides (abbreviated A, G, C and T). However, by "nucleotide sequence" of a nucleic acid molecule or polynucleotide is intended, for a DNA molecule or polynucleotide, a sequence of deoxyribonucleotides, and for an RNA molecule or polynucleotide, the corresponding sequence of ribonucleotides (A, G, C and U) where each thymidine deoxynucleotide (T) in the specified deoxynucleotide sequence in is replaced by the ribonucleotide uridine (U). For instance, reference to an RNA molecule having the sequence of SEQ ID NO:1 set forth using deoxyribonucleotide abbreviations is intended to indicate an RNA molecule having a sequence in which each deoxynucleotide A, G or C of SEQ ID NO:1 has been replaced by the corresponding ribonucleotide A, G or C, and each deoxynucleotide T has been replaced by a ribonucleotide U.

[0071] The present invention is also directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated DNA molecule having the nucleotide sequences shown in Table 6 is intended DNA fragments at least 15 nucleotides, at least 20 nucleotides, at least 30 nucleotides in length, which are useful as diagnostic probes and primers is discussed in more detail below. Of course larger nucleic acid fragments of up to the entire length of the nucleic acid molecules of the present invention are also useful diagnostically as probes, according to conventional hybridization techniques, or as primers for amplification of a target sequence by the polymerase chain reaction (PCR), as described, for instance, in Molecular Cloning, A Laboratory Manual, 3rd. edition, edited by Sambrook, J and Russel, D.W., (2001), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, the entire disclosure of which is hereby incorporated herein by reference.

[0072] By a fragment at least 20 nucleotides in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the as shown in Table 6. The nucleic acids containing the nucleotide sequences listed in Table 6 can be generated using conventional methods of DNA synthesis which will be routine to the skilled artisan. For example, restriction endonuclease cleavage or shearing by sonication could easily be used to generate fragments of

various sizes. Alternatively, the DNA fragments of the present invention could be generated synthetically according to known techniques.

[0073] In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above. By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides, at least about 20 nucleotides, at least about 30 nucleotides, and more than 30 nucleotides of the reference polynucleotide. These fragments that hybridize to the reference fragments are useful as diagnostic probes and primers. A probe, as used herein is defined as at least about 100 contiguous bases of one of the nucleic acids in Table 6. For the purpose of the invention, two sequences hybridize when they form a double-stranded complex in a hybridization solution of 6X SSC, 0.5% SDS, 5X Denhardt's solution and 100μg of non-specific carrier DNA. See Ausubel *et al.*, section 2.9, supplement 27 (1994). Sequences may hybridize at "moderate stringency," which is defined as a temperature of 60 °C in a hybridization solution of 6X SSC, 0.5% SDS, 5X Denhardt's solution and 100μg of non-specific carrier DNA. For "high stringency" hybridization, the temperature is increased to 68 °C. Following the moderate stringency hybridization reaction, the nucleotides are washed in a solution of 2X SSC plus 0.05% SDS for five times at room temperature, with subsequent washes with 0.1X SSC plus 0.1% SDS at 60 °C for 1h. For high stringency, the wash temperature is increased to 68 °C. For the purpose of the invention, hybridized nucleotides are those that are detected using 1 ng of a radiolabeled probe having a specific radioactivity of 10,000 cpm/ng, where the hybridized nucleotides are clearly visible following exposure to X-ray film at -70 °C for no more than 72 hours.

[0074] As mentioned previously, the present application is directed to such nucleic acid molecules which are at least 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to a nucleic acid sequence described above. One embodiment encompasses

nucleic acid molecules which are at least 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequence shown in Table 6. By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence, is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

[0075] As a practical matter, whether any particular nucleic acid molecule is at least 95%, 96%, 97%, 98% or 99% identical to a reference nucleotide sequence refers to a comparison made between two molecules using standard algorithms well known in the art. Although any sequence algorithm can be used to define sequence identity, for clarity, the present invention defines identity with reference to the Basis Local Alignment Search Tool (BLAST) algorithm (Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990)), where a promoter sequence set forth in the disclosure is used as the reference sequence to define the percentage identity of polynucleotide homologues over its length. The choice of parameter values for matches, mismatches, and inserts or deletions is arbitrary, although some parameter values have been found to yield more biologically realistic results than others.

[0076] When using BLAST or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide

sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

[0077] Relatedness between two polynucleotides also may be described by reference to their ability to hybridize to form double-stranded complexes by the formation of complementary base pairs. Hybridization conditions have been described previously herein. An increase in temperature can be used to break apart these complexes. The more structurally identical two sequences are, the higher the temperature required to break them apart or "melt" them. The temperature required to melt a double-stranded complex is called the "T_m." The relationship between the T_m and other hybridization parameters is given by:

$$T_m\,(^\circ C) = 81.5 + 16.6(\log_{10}[Na^+]) + 0.41(\text{fraction } G + C) - 0.63\,(\%\text{ formamide}) - (600/l),$$

where T_m is the melting temperature of a DNA duplex consisting of the probe and its target; and l = the length of the hybrid in base pairs, provided $l > 100$ base pairs. Bolton *et al.*, *Proc. Natl. Acad. Sci.* **48**:1390 (1962). Generally, a change of 1°C in the melting point represents from 0.7% to 3.2% difference in DNA sequence similarity. Bonner *et al.*, *Journal of Molecular Biology* **81**:123-35 (1973); McCarthy *et al.*, *In* EVOLUTION OF GENETIC SYSTEMS, H.H. Smith (ed.), Brookhaven Symposium in Biology No. 23, Gordon and Breach, New York, pp. 1-43 (1972). The formation of a stable DNA duplex at 60 °C typically requires at least an 80% sequence identity between sequences. Sibley *et al.*, *ACTA* **1**: 83-121 (Proceedings of the 18th International Ornithological Congress, Moscow, August 16-24, 1982, Academy of Sciences of the USSR).

[0078] The nucleic acids of the present invention confer preferential expression of polypeptides or proteins in the vascular tissue of plants. The nucleic acids of the present invention can also preferentially direct the expression of antisense RNA, or RNA involved in RNA interference (RNAi) such as small interfering RNA (siRNA), in the vascular tissue of plants, which can be useful for inhibiting or completely blocking the expression of targeted genes.

[0079] As used herein, vascular plant tissue refers to xylem, phloem or vascular cambium tissue. In one embodiment, the promoters of the invention are either "**xylem-specific**", "**cambium-specific**" and/or "**phloem-specific**" and direct expression of an operably linked nucleic acid segment in the xylem, cambium and/or phloem, respectively. As used herein, "**coding product**" is intended to mean the ultimate product of the nucleic acid that is operably linked to the promoters. For example, a protein or polypeptide is a coding product, as well as antisense RNA or siRNA which is the ultimate product of the nucleic acid coding for the antisense RNA. The coding product may also be non-translated mRNA. The terms polypeptide and protein are used interchangeably herein. **Xylem-specific**, for example, is intended to mean that the nucleic acid molecules of the current invention are more active in the xylem than in any other plant tissue. In one embodiment, the nucleic acids of the current invention are promoters that are active specifically in the xylem, cambium and/or phloem, meaning that the promoters are only active in the xylem, cambium, and/or phloem tissue of plants, respectively. In other words, a "**xylem-specific**" promoter, for example, drives the expression of a coding product such that detectable levels of the coding product are expressed only in xylem tissue of a plant. However, because of solute transport in plants, the coding product that is specifically expressed in the xylem, phloem or cambium may be found anywhere in the plant and thus its presence is not necessarily confined to xylem tissue. A **vascular-preferred promoter**, on the other hand can be preferentially active is any of the xylem, phloem or cambium tissues, or in at least two of the three tissue types. A **vascular-specific promoter**, is specifically active in any of the xylem, phloem or cambium, or in at least two of the three tissue types.

[0080] The **vascular system**, as used herein, comprises the xylem and phloem tissues that are used to conduct water and photosynthates in higher plants and the vascular cambium that is positioned between the xylem and phloem. As components of the vascular system, xylem and phloem are collectively referred to as vascular tissue. Vascular tissue is produced during two developmentally distinct stages. During embryogenesis and/or the post-embryogenic stage, primary vascular tissue

develops from the differentiation of the apical meristem and the procambium. **Secondary vascular tissue** develops from the differentiation of the vascular cambium

[0081] The **vascular cambium** is the lateral meristem positioned between the xylem and phloem and differentiates to produce secondary vascular tissue. The vascular cambium is composed of two cell types, **fusiform** and **ray initials**, which are collectively referred to as **cambial initials**. The division of fusiform initials results in the longitudinal or axial systems of the xylem and phloem, whereas the division of ray initials leads to the development of the transverse or ray systems. The cambial initials divide about a tangential axis, such that the xylem cells aggregate towards the interior of the axis and the phloem cells towards the periphery.

[0082] As used herein, **xylem** refers to the conducting tissue that transports water and mineral ions from the root system to aerial portions of the plant. Both primary and secondary xylem tissue comprises three distinct cell types, the **tracheary elements, fibers**, and **parenchyma cells**. The tracheary elements, comprising **imperforate tracheids** and **perforate vessel elements**, function in water conduction. **Xylem fibers**, comprising **fiber tracheids** and **libriform fibers**, impart strength and rigidity to the xylem structure. **Parenchyma cells** are comprised of **axial** and **ray parenchyma cells**, which are important for the storage of starch, oil, and other energy-rich molecules. Thus, a **xylem-specific promoter**, as contemplated in the current invention, is active in the tracheary elements, fibers, and/or parenchyma cells of the primary and/or secondary xylem.

[0083] **Phloem** is the plant tissue that translocates the products of photosynthesis from mature leaves to areas of growth and storage. The principal cells comprising both primary and secondary phloem tissue include **sieve elements, parenchyma cells**, and **fibers**. The sieve elements refer to the conducting elements of the phloem and are further comprised of **sieve cells** in gymnosperms or **sieve-tube elements** present in angiosperms. **Sieve-tube elements** form specialized structures called **sieve tubes**, wherein the sieve tube is comprised of a series of sieve-tube elements that are vertically arranged and interconnect through a portion of the cell wall referred to as

the sieve plate. In contrast, the less specialized sieve cells, present in gymnosperms and lower vascular plants, do not interconnect to form **sieve plates**. Sieve cells are arranged in overlapping arrays such that conduction is facilitated through enlarged pores, called **sieve areas**. The sieve elements are associated with, and depend upon, specialized parenchyma cells called **companion cells** in angiosperms and **albuminous cells** in gymnosperms. Both companion and albuminous cells provide storage for proteins and metabolites necessary for sieve element function. In addition to the specialized parenchyma cells, the phloem contains **parenchyma cells** that function in the accumulation and storage of starch, fat, and other compounds. **Phloem fibers** impart strength to the phloem structure and may contribute to starch storage. Thus, a **phloem-specific promoter**, as contemplated in the present invention, is active in the sieve elements, parenchyma cells, and/or fibers of the primary and/or secondary phloem.

[0084] In most higher plants, the vascular tissues appear as a cylinder positioned between ground tissue. In both the stem and root, the **pith** and **cortex** comprise the ground tissue. The pith is located in the interior of vascular cylinder, whereas the cortex is located between the epidermis and the vascular tissue. Collectively, the vascular system and associated ground tissues are termed the **vascular cylinder.**

[0085] As used herein, promoter is intended to mean a nucleic acid, preferably DNA, that binds RNA polymerase and/or other transcription regulatory elements. As with any promoter, the promoters of the current invention will facilitate or control the transcription of DNA or RNA to generate an mRNA molecule from a nucleic acid molecule that is operably linked to the promoter. As stated earlier, the RNA generated may code for a protein or polypeptide or may code for an RNA interfering, or antisense molecule. As used herein, "operably linked" is meant to refer to the chemical fusion, ligation, or synthesis of DNA such that a promoter-nucleic acid sequence combination is formed in a proper orientation for the nucleic acid sequence to be transcribed into an RNA segment. The promoters of the current invention may also contain some or all of the 5' untranslated region (5' UTR) of the resulting mRNA

transcript. On the other hand, the promoters of the current invention do not necessarily need to possess any of the 5' UTR.

[0086] A promoter, as used herein, may also include regulatory elements. Conversely, a regulatory element may also be separate from a promoter. Regulatory elements confer a number of important characteristics upon a promoter region. Some elements bind transcription factors that enhance the rate of transcription of the operably linked nucleic acid. Other elements bind repressors that inhibit transcription activity. The integrated effect of transcription factors on promoter activity may determine whether the promoter activity is high or low, i.e. whether the promoter is "strong" or "weak." Transcription factors that bind regulatory elements may themselves be regulated by the interaction with other bound proteins or by covalent modification, e.g. phosphorylation, in response to extracellular stimuli. The activity of some transcription factors is modulated by signaling molecules, such as intracellular metabolites or chemicals exogenous to the organism that communicate with the cellular nucleus. Promoters that are unaffected by changes in the cellular environment are referred to as constitutive promoters.

[0087] The present invention also provides vectors comprising the isolated nucleic acid molecules of the invention. In one embodiment, the vectors of the present invention are Ti-plasmids derived from the *A. tumefaciens.*

[0088] In developing the nucleic acid constructs of this invention, the various components of the construct or fragments thereof will normally be inserted into a convenient cloning vector, e.g., a plasmid that is capable of replication in a bacterial host, e.g., *E. coli.* Numerous vectors exist that have been described in the literature, many of which are commercially available. After each cloning, the cloning vector with the desired insert may be isolated and subjected to further manipulation, such as restriction digestion, insertion of new fragments or nucleotides, ligation, deletion, mutation, resection, etc. to tailor the components of the desired sequence. Once the construct has been completed, it may then be transferred to an appropriate vector for further manipulation in accordance with the manner of transformation of the host cell.

[0089] A recombinant DNA molecule of the invention typically includes a selectable marker so that transformed cells can be easily identified and selected from non-transformed cells. Examples of such markers include, but are not limited to, a neomycin phosphotransferase (*nptII*) gene (Potrykus et al. (1985), Mol. Gen. Genet. 199:183-188), which confers kanamycin resistance. Cells expressing the *nptII* gene can be selected using an appropriate antibiotic such as kanamycin or G418. Other commonly used selectable markers include the bar gene, which confers bialaphos resistance; a mutant EPSP synthase gene (Hinchee et al., Bio/Technology 6:915-922 (1988)), which confers glyphosate resistance; a nitrilase gene, which confers resistance to bromoxynil (Stalker et al. (1988) J. Biol. Chem. 263:6310-6314); a mutant acetolactate synthase gene (ALS), which confers imidazolinone or sulphonylurea resistance (European Patent Application 154,204, 1985); and a methotrexate resistant DHFR gene (Thillet et al. (1988) J. Biol. Chem. 263:12500-12508).

[0090] Additionally, vectors may include an origin of replication (replicons) for a particular host cell. Various prokaryotic replicons are known to those skilled in the art, and function to direct autonomous replication and maintenance of a recombinant molecule in a prokaryotic host cell.

[0091] The vectors will preferably contain selectable markers. Numerous selectable markers for use in selecting transfected plant cells including, but not limited to, kanamycin, glyphosate resistance genes, and tetracycline or ampicillin resistance for culturing in *E. coli, A. tumefaciens* and other bacteria.

[0092] A plasmid vector suitable for the introduction of nucleic acid of the current invention into monocots using microprojectile bombardment is composed of the following: the promoter of choice; an intron that provides a splice site to facilitate expression of the gene, such as the Hsp70 intron (PCT Publication WO 93/19189); and a 3' polyadenylation sequence such as the nopaline synthase 3' sequence (NOS 3'; Fraley et al. (1983) Proc Natl Acad Sci USA 80: 4803-4807). This expression

cassette may be assembled on high copy replicons suitable for the production of large quantities of DNA.

[0093] A particularly useful *Agrobacterium*-based plant transformation vector for use in transformation of dicotyledonous plants is plasmid vector pMON530 (Rogers et al. (1987) Improved vectors for plant transformation: expression cassette vectors and new selectable markers. In Methods in Enzymology. Edited by R. Wu and L. Grossman. p253-277. San Diego: Academic Press). Plasmid pMON530 is a derivative of pMON505 prepared by transferring the 2.3 kb StuI-HindIII fragment of pMON316 (Rogers et al. (1987) Improved vectors for plant transformation: expression cassette vectors and new selectable markers. In Methods in Enzymology. Edited by R. Wu and L. Grossman. p253-277. San Diego: Academic Press) into pMON526. Plasmid pMON526 is a simple derivative of pMON505 in which the SmaI site is removed by digestion with XmaI, treatment with Klenow polymerase and ligation. Plasmid pMON530 retains all the properties of pMON505 and the CaMV35S-NOS expression cassette and now contains a unique cleavage site for SmaI between the promoter and polyadenylation signal.

[0094] Binary vector pMON505 is a derivative of pMON200 (Rogers et al., 1987) in which the Ti plasmid homology region, LIH, has been replaced with a 3.8 kb HindIII to SmaI segment of the mini RK2 plasmid, pTJS75 (Schmidhauser and Helinski. (1985) J. Bacteriol. 164-155). This segment contains the RK2 origin of replication, oriV, and the origin of transfer, oriT, for conjugation into *Agrobacterium* using the tri-parental mating procedure (Horsch and Klee. (1986) Proc. Natl. Acad. Sci. U. S. A. 83:4428-4432). Plasmid pMON505 retains all the important features of pMON200 including the synthetic multi-linker for insertion of desired DNA fragments, the chimeric NOS/NPTII'/NOS gene for kanamycin resistance in plant cells, the spectinomycin/streptomycin resistance determinant for selection in *E. coli* and *A. tumefaciens*, an intact nopaline synthase gene for facile scoring of transformants and inheritance in progeny, and a pBR322 origin of replication for ease in making large amounts of the vector in *E. coli*. Plasmid pMON505 contains a single T-DNA border derived from the right end of the pTiT37 nopaline-type T-DNA.

Southern blot analyses have shown that plasmid pMON505 and any DNA that it carries are integrated into the plant genome, that is, the entire plasmid is the T-DNA that is inserted into the plant genome. One end of the integrated DNA is located between the right border sequence and the nopaline synthase gene and the other end is between the border sequence and the pBR322 sequences.

[0095] Another particularly useful Ti plasmid cassette vector is pMON17227. This vector is described in PCT Publication WO 92/04449 and contains a gene encoding an enzyme conferring glyphosate resistance (denominated CP4), which is an excellent selection marker gene for many plants, including potato and tomato. The gene is fused to the *Arabidopsis* EPSPS chloroplast transit peptide (CTP2), and expression is driven by the promoter of choice.

[0096] For secretion of the translated protein into the lumen of the endoplasmic reticulum, the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. The signals may be endogenous to the polypeptide or they may be heterologous signals.

[0097] In one embodiment, the vectors of the current invention are designed in a manner such that the nucleic acids described herein are tissue-specific promoters which are operably linked to DNA encoding a polypeptide of interest. In another embodiment, the polypeptide of interest is an enzyme involved in lignin biosynthesis in plants. Polynucleotides encoding many of the enzymes involved in lignin biosynthesis include, but are not limited to, cinnamyl alcohol dehydrogenase (CAD), cinnamate 4-hydroxylase (C4H), coumarate 3-hydroxylase (C3H), phenolase (PNL), O-methyl transferase (OMT), cinnamoyl-CoA reductase (CCR), phenylalanine ammonia-lyase (PAL), 4-coumarate:CoA ligase (4CL) and peroxidase (POX) from pine. U.S. Pat. No. 6,204,434. Other enzymes include coniferin β-glucosidase (CBG), and caffeic acid 3-O-methyltransferase (COMT). U.S. Pat. No. 5,451,514, WO 94/23044, and Dharmawardhana *et al.*, *Plant Mol. Biol.* **40**: 365-72 (1999).

[0098] In another embodiment, the coding sequence operably linked to the promoter may code for a gene product that inhibits the expression or activity of enzymes

involved in lignin biosynthesis. For example, of particular interest for control of lignin biosynthesis is an antisense gene for cinnamyl alcohol dehydrogenase (CAD), cinnamate 4-hydroxylase (C4H), coumarate 3-hydroxylase (C3H), phenolase (PNL), O-methyl transferase (OMT), cinnamoyl-CoA reductase (CCR), phenylalanine ammonia-lyase (PAL), 4-coumarate:CoA ligase (4CL) and peroxidase (POX) from pine.

[0099] In a further embodiment, the vectors of the current invention are designed such that the nucleic acids of the current invention are operably linked to DNA or RNA that encodes antisense RNA or interfering RNA, which corresponds to genes that code for polypeptides of interest, resulting in a decreased expression of targeted gene products. Preferably the gene products targeted for suppression are enzymes involved in lignin biosynthesis, as discussed previously. The use of RNAi inhibition of gene expression is described generally in Paddison *et al.*, *Genes & Dev.* 16: 948-958 (2002), and the use of RNAi to inhibit gene expression in plants is specifically described in WO 99/61631, both of which are herein incorporated by reference.

[0100] The use of antisense technology to reduce or inhibit the expression of specific plant genes has been described, for example in European Patent Publication No. 271988. Reduction of gene expression led to a change in the phenotype of the plant, either at the level of gross visible phenotypic difference, for example a lack of lycopene synthesis in the fruit of tomato leading to the production of yellow rather than red fruit, or at a more subtle biochemical level, for example, a change in the amount of polygalacturonase and reduction in depolymerisation of pectins during tomato fruit ripening (Smith et. al., Nature, 334:724-726 (1988); Smith et. al., Plant Mol. Biol., 14:369-379 (1990)). Thus, antisense RNA has been demonstrated to be useful in achieving reduction of gene expression in plants.

[0101] In one embodiment of the method of making a plant of the invention, an exogenous DNA capable of being transcribed inside a plant to yield an antisense RNA transcript is introduced into the plant, e.g., into a plant cell. The exogenous DNA can be prepared, for example, by reversing the orientation of a gene sequence with respect

to its promoter. Transcription of the exogenous DNA in the plant cell generates an intracellular RNA transcript that is "antisense" with respect to that gene.

[0102] For example, antisense technology may be used to suppress the expression of gene encoding enzymes involved in lignin biosynthesis, such as 4CL. A construct containing an antisense 4CL DNA sequence can be prepared by reversing the orientation of an exogenous DNA sequence encoding 4CL and operably linking the sequence to a promoter. Transcription of the exogenous 4CL DNA sequence in the plant cell would generate a 4CL RNA transcript that is in the antisense orientation.

[0103] The invention also provides host cells which comprise the vectors of the current invention. As used herein, a host cell refers to the cell in which the coding product is ultimately expressed. Accordingly, a host cell can be an individual cell, a cell culture or cells as part of an organism. The host cell can also be a portion of an embryo, endosperm, sperm or egg cell, or a fertilized egg.

[0104] The polynucleotides or vectors containing the polynucleotides of the current invention are introduced into the host cells by standard procedures known in the art for introducing recombinant vector DNA into the target host cell. Such procedures include, but are not limited to, transfection, infection, transformation, natural uptake, electroporation, biolistics and *Agrobacterium*. Methods for introducing foreign genes into plants are known in the art and can be used to insert a gene construct of the invention into a plant host, including, biological and physical plant transformation protocols. See, for example, Miki et al., 1993, "Procedure for Introducing Foreign DNA Into Plants", In: Methods in Plant Molecular Biology and Biotechnology, Glick and Thompson, eds., CRC Press, Inc., Boca Raton, pages 67-88. The methods chosen vary with the host plant, and include chemical transfection methods such as calcium phosphate, microorganism-mediated gene transfer such as *Agrobacterium* (Horsch et al., Science 227:1229-31, 1985), electroporation, micro-injection, and biolistic bombardment.

[0105] Accordingly, the present invention also provides plants or plant cells, comprising the polynucleotides or vectors comprising the polynucleotides of the

current invention. In one embodiment, the plants are angiosperms or gymnosperms. In another embodiment, the plants are selected from *Eucalyptus* and *Pinus* species. In particular, the transgenic plant may be of the species *Eucalyptus grandis* and hybrids, *Pinus radiata*, *Pinus taeda* L (loblolly pine), *Populus nigra*, *Populus deltoides*, or *Liquidamber styraciflua*. Beyond the ordinary meaning of plant, the term "plants" is also intended to mean the fruit, seeds, flower, strobilus etc. of the plant. The plant of the current invention may be a direct transfectant, meaning that the vector was introduced directly into the plant, such as through *Agrobacterium*, or the plant may be the progeny of a transfected plant. The progeny may also be obtained by asexual reproduction of a transfected plant. The second or subsequent generation plant may or may not be produced by sexual reproduction, i.e., fertilization. Furthermore, the plant can be a gametophyte (haploid stage) or a sporophyte (diploid stage).

[0106] The present invention also provides a method of controlling the lignin content of a plant comprising cultivating a plant or seed comprising the vectors of the current invention. Proper cultivation to induce or sustain the growth or germination of the plants or seeds of the current invention is species-specific, and within the level of ordinary skill in the art. The setting for cultivation may be anywhere which fosters the growth or germination of the plant or seed. Furthermore, cultivation can also include steps such as, but not limited to, providing a stress treatment, (e.g., nitrogen deprivation, heat shock, low temperatures, sucrose deprivation) which can induce embyrogenesis in anthers and/or microspores.

[0107] The invention further provides isolated regulatory elements that bind transcription factors and are capable of regulating tissue-preferred or tissue-specific expression. The degree of regulation conferred by the regulatory elements may be complete, meaning that transcription is not detectable without the transcription factors, or partial, meaning that transcription is enhanced in the presence of the transcription factors. In one embodiment, at least one regulatory element is operably linked to a heterologous promoter to provide a composite promoter. The composite promoter is expressed preferentially or specifically in vascular tissue. As used herein, heterologous promoters is a phrase whose meaning term that is relative to the

regulatory elements. If a regulatory element and a promoter do not associate with one another in a natural setting, the promoter would be considered heterologous to the regulatory element. Typically, the precise orientation of a regulatory element within a promoter region will not affect its activity. Furthermore, regulatory elements can function normally when inserted into heterologous promoter regions. Thus, for example, xylem-preferred regulatory elements can be removed from their endogenous promoter and can be inserted into heterologous promoter regions to confer xylem-specificity or preference. Likewise, phloem-preferred regulatory elements can be removed from their endogenous promoter and can be inserted into heterologous promoter regions to confer phloem-specificity or preference. Similarly, cambium-preferred regulatory elements can be removed from their endogenous promoter and can be inserted into heterologous promoter regions to confer cambium-specificity or preference. The heterologous promoter may be, for example, a minimal CaMV 35S promoter. Promoters that direct expression in plant cells which are suitable for modification to minimal promoters include the cauliflower virus (CaMV) 35S promoter (Jefferson *et al.*, *EMBO J.*, 6: 3901-07 (1987)), the rice actin promoter (McElroy *et al.*, *Plant Cell*, 2: 163-71 (1990)), the maize ubiquitin-1 promoter (Christensen *et al.*, *Transgenic Research*, 5: 213-18 (1996)), and the nopaline synthase promoter (Kononowics *et al.*, *Plant Cell* 4: 17-27 (1992)).

[0108] To prepare the nucleic acids of the invention, genomic libraries were made from *Pinus radiata* and *Eucalyptus grandis*, using a variety of restriction endonucleases to digest the genome into discrete fragments. Genomic libraries can be similarly constructed from any plant species from which it is desirable to obtain tissue-selective promoters. An adaptor was ligated to each of these genomic sequences, according to the procedure provided by Clontech for use of its GenomeWalker™ Systems (Clontech, Palo Alto, CA). Promoter sequences then were PCR-amplified using adaptor-specific primers and "gene-specific primers." Alternatively, this PCR amplification step optionally may be conducted by the methodology described in U.S. Patents No. 5,565,340 and No. 5,759,822, herein incorporated by reference, to yield reaction products of long length and minimal background. Using this general PCR amplification methodology, the identification of

the promoter of the invention and its identification as a tissue-selective promoter, is governed by the choice of the "gene-specific primer."

[0109] A gene-specific primer is any transcribed sequence that is expressed at high levels in a tissue of interest. In the present invention, the gene-specific primer is a fragment of, or is complementary to, an mRNA that is expressed at high levels in vascular tissue. In one embodiment, the gene-specific primer is selected by its homology to genes that are known to be expressed specifically in a particular vascular tissue type. Genes of particular interest are those that are expressed in a particular vascular tissue at high levels, which typically is an indicator of vascular-selective activity of the corresponding promoter.

[0110] Expressed sequence tags (ESTs) provide another source of gene-specific primers. An EST is a cDNA fragment of a corresponding mRNA that is present in a given library. Any plant EST database may be searched electronically to find ESTs that share identity to segments of genes that are known to be expressed specifically in a desired tissue type ("in silico screening"). These ESTs thus will provide gene-specific primers for the amplification of the promoter of the corresponding gene in a given genomic library. The amplified gene promoter need not be from the same species from which the EST database was obtained. All that is required is that the EST bears sufficient sequence similarity to the gene promoter of interest to act as a primer for PCR amplification of the target segment of the gene.

[0111] An alternative methodology to identify tissue-specific promoters rests on detection of mRNAs that are expressed in one tissue type, but not in another, implying that they are transcribed from a tissue-specific promoter. Populations of mRNAs can be distinguished on this basis by subtractive hybridization, for example. One such suitable subtractive hybridization technique is the PCR-Select™ described by Clontech.

[0112] Alternatively, a tissue-specific mRNA distribution can be determined by *in situ* hybridization of thin slices of plant tissue with radiolabeled probes. Probes that radioactively stain a particular tissue type are then used to detect the promoter

associated with the mRNA by Southern analysis of genomic libraries, using the methodologies described below. All of the aforementioned techniques require the preparation of mRNA libraries from the tissue of interest, in this case vascular tissue. The preparation of cDNA libraries from xylem tissue, for example, is described in Dharmawardhana et al., *Plant Mol. Biol.* **40**: 365-72 (1999) or Loopstra et al., *Plant Mol. Biol.* **27**: 277-91 (1995). Briefly, actively differentiating xylem is first stripped from felled trees. Total RNA is isolated using standard techniques, and poly(A) RNA then is isolated and reverse transcribed to construct a xylem-tissue cDNA library. As described in Dharmawardhana (1999), for example, the cDNA library was constructed in the λZAP-XR vector, employing Strategene cDNA synthesis and GigapakII Gold™ packaging kits. Xylem-specific promoters can, in turn, be isolated from such cDNA libraries by PCR using a gene-specific probe and a primer that recognizes a sequence at the 5' end of the promoter. A gene-specific probe can be obtained by the *in silico* approach described above, or by designing a specific probe based on the sequence of the mRNA, if known. Furthermore, a primer can be synthesized which is complementary to the 5' UTR of the desired target gene. Alternatively, the primer can be designed from a partial amino acid sequence of the encoded protein, as a so-called degenerate primer. (See Dharmawardhana (1999)).

[0113] Following isolation of the promoter of interest, various methods can be used to characterize its tissue-specific expression pattern and promoter strength. One commonly employed method is to operably link the promoter to a readily assayed reporter gene. For example, the xylem-preferred promoter and 5' UTR of the *Eucalyptus gunnii* cinnamoyl-CoA reductase (EgCCR) gene (position -1448 to +200, where +1 is the transcription start site) has been operably linked to the gene encoding β-glucuronidase (GUS). Lacombe et al., *Plant J.* **23**: 663-76 (2000). Suitable expression constructs can be made using well-known methodologies.

[0114] Transformation of plants can be accomplished by any one of many suitable techniques, including *Agrobacterium*-mediated transformation, as described in U.S. Patent No. 6,051,757. Other methods for transforming trees are known in the art, as exemplified by U.S. Patent No. 6,518,485, which discloses an accelerated particle

transformation method of gymnosperm somatic embryos. Other transformation methods include micro-projectile bombardment (Klein et al., *Biotechnology* **6**: 559-63 (1988)), electroporation (Dhalluin et al., *Plant Cell* **4**: 1495-1505 (1992)), and polyethylene glycol treatment (Golovkin et al., *Plant Sci.* **90**: 41-52 (1993)). Further, U.S. Patent No. 6,187,994 provides for a recombinase-assisted insertion of the expression construct into a specific, selected site within a plant genome. All of the aforementioned patents and publications are herein incorporated by reference.

[0115] A DNA molecule of the present invention can be inserted into the genome of a plant by any suitable method. Suitable plant transformation vectors include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed, e.g., by Herrera-Estrella et al. (1983) *Nature* 303:209, Bevan (1984) *Nucleic Acids Res.* 12 (22): 8711-8721, Klee et al. (1985) *Bio/Technology* 3(7): 637-642 and EPO publication 120,516. In addition to plant transformation vectors derived from the Ti or root-inducing (Ri) plasmids of *Agrobacterium*, alternative methods can be used to insert the DNA constructs of this invention into plant cells. Such methods may involve, for example, the use of liposomes, electroporation, chemicals that increase free DNA uptake, free DNA delivery via microprojectile bombardment, and transformation using viruses or pollen. DNA may also be inserted into the chloroplast genome (Daniell et al. (1998) *Nature Biotechnology* 16:345-348).

[0116] When adequate numbers of cells (or protoplasts) containing the nucleic acid of interest are obtained, the cells (or protoplasts) are regenerated into whole plants. Choice of methodology for the regeneration step is not critical, with suitable protocols being available for hosts from *Leguminosae* (alfalfa, soybean, clover, etc.), *Umbelliferae* (carrot, celery, parsnip), *Cruciferae* (cabbage, radish, canola/rapeseed, etc.), *Cucurbitaceae* (melons and cucumber), *Gramineae* (wheat, barley, rice, maize, etc.), *Solanaceae* (potato, tobacco, tomato, peppers), various floral crops, such as sunflower, and nut-bearing trees, such as almonds, cashews, walnuts, and pecans. See, e.g., Ammirato *et al.* (1984) Handbook of Plant Cell Culture-Crop Species. Macmillan Publ. Co.; Fromm, M., (1990) UCLA Symposium on Molecular Strategies for Crop Improvement, Apr. 16-22, 1990. Keystone, CO.; Vasil et al. (1990)

Bio/Technology 8:429-434; Vasil *et al.* (1992) *Bio/Technology* 10:667-674; Hayashimoto et al. (1990) *Plant Physiol.* 93:857-863; and Datta *et al.* (1990).

[0117] The vector comprising the promoter and reporter gene includes a mechanism to select those plant cells successfully transformed with the vector, which may be, for example, kanamycin resistant. The presence of the GUS gene in transformants may be confirmed by a PCR approach, using GUS-specific PCR primers (Clontech, Palo Alto). Segregation of kanamycin resistance in the progeny of the transformed plant cells can be used in conjunction with Southern analysis to determine the number of loci harboring the stably inserted vector. The temporal and spatial pattern of promoter expression is then inferred from a quantification of the reporter gene expression, as described in Jefferson et al., *EMBO J.* **6**: 3901-07 (1987). Generally, GUS expression is determined histochemically in thin slices of plant tissues that are fixed first in 90% acetone and then in a buffered solution containing a GUS substrate, 5-bromo-4-chloro-3-indoyl-β-D-glucuronic acid (X-Gluc). The presence of the GUS expression product is indicated by a colorimetric reaction with the X-Gluc.

[0118] Xylem-specific expression, for example, can be conferred by the presence of regulatory elements that specifically bind transcription factors in xylem tissue. The interaction between transcription factors that influence the degree of xylem specific expression depends on the alignment between a subset of base pairs of the regulatory element with amino acid residues of the transcription factor. Likewise, phloem-specific or cambium-specific expression, for example, can be conferred by the presence of regulatory elements that specifically bind transcription factors in phloem and cambium tissue, respectively. Base pairs that do not interact with the bound transcription factor may be substituted with other base pairs, while maintaining the overall ability of the regulatory element to bind specifically the tissue-specific transcription factor.

[0119] Various methodologies can be used to identify and characterize regulatory elements that affect tissue-preferred or tissue-specific promoter activity, once a promoter is identified as tissue-preferred or specific. In one methodology, the

promoter region is sequentially truncated at the 5' end and the series of truncated promoters are each operably linked to a reporter gene. When a regulatory element is deleted, the effect on the promoter activity is inferred by the loss of tissue-specific expression of the reporter gene. Alternatively, a putative regulatory element can be inserted into an expression construct containing a minimal promoter, such as the CaMV 35S minimal promoter (Keller *et al.*, *Plant Mol. Biol.* **26**: 747-56) to ascertain if the putative regulatory element confers tissue-specific expression. A minimal promoter contains only those elements absolutely required for promoter activity, such as a RNA polymerase binding site. Additional examples for elucidating putative regulatory elements are provided by studies of tissue-specific regulatory elements that coordinately regulate transcription of the genes encoding L-phenyl-alanine ammonia-lyase (PAL) and 4-coumarate CoA ligase (4CL). Hatton *et al.*, *Plant J.* **7**: 859-76 (1995); Leyva *et al.*, *Plant Cell* **4**: 263-71 (1992); Hauffe *et al.*, *Plant J.* **4**: 235-53 (1993); Neustaedter *et al.*, *Plant J.* **18**: 77-88 (1999), all of which are incorporated herein by reference.

[0120] Another methodology to locate putative regulatory elements is to compare sequences among known vascular-preferred or vascular-specific regulatory elements. Nucleotide comparison will identify regions similar to known tissue-preferred regulatory elements, such as, for example, vascular-preferred regulatory elements. For example, comparison of promoter sequences between cinnamyl CoA reductase and other promoters involved in phenyl-propanoid production reveals a conserved AC-rich region having the sequence CCCACCTACC. *See* Lacombe (2000). Conservation of the above-noted sequence in promoters involved in phenylpropanoid synthesis implies that the conserved sequence is a binding site for a coordinately activating transcription factor, such as MYB, which has been identified in several plant species. *See* Martin, *Trends Genet.* **13**: 67-73 (1997). For example, MYB binding sites have been identified in maize ([C/A]TCC[T/A]ACC) and Petunia (TAAC[C/G]GTT or TAACTAAC). Id.

[0121] Sequence comparisons of this sort, based on the nucleic acid sequences of the invention, reveal a series of motifs shown in Table 1, below. It is expected that

the motifs in Table 1 bind tissue-preferred or tissue-specific transcription factors and can be used to modulate tissue-preferred or tissue-specific expression of a heterologous, minimal promoter.

TABLE 1

SEQ ID NO:	MOTIF	PROMOTER
SEQ ID NO: 72	AATCAAATCCTCC	Eucalyptus Microtubule assoc (SEQ ID NO 5)
SEQ ID NO: 73	AATCAAATCCTCC	Eucalyptus F5H (SEQ ID NO 7)
SEQ ID NO: 74	TCTCCCTCCTCT	Eucalyptus cellulose synthase (SEQ ID NO 8)
SEQ ID NO: 75	ATAAAGAAGTGAA	Eucalyptus cellulose synthase SEQ ID NO 8)
SEQ ID NO: 76	TAAACTTATTTTCT	Pine LIM (SEQ ID NO 9)
SEQ ID NO: 77	TAAACTTATTTTCT	Pine Pectate Lyase (SEQ ID NO 10)
SEQ ID NO: 78	GGAGAAACAAAA	Pine Pectate Lyase (SEQ ID NO 10)
SEQ ID NO: 79	AAGTAACCAATGATGC	Pine Expansin (SEQ ID NO 11)
SEQ ID NO: 80	ACTTTGAAGAAAA	Pine Expansin (SEQ ID NO 11)
SEQ ID NO: 81	TGAGGAGAAGA	Pine 4CL3 (SEQ ID NO 13)
SEQ ID NO: 82	ATCAAGCTGAT	Pine 4CL (SEQ ID NO 13)
SEQ ID NO: 83	AATTTCATTTTC	Pine dirigent (SEQ ID NO 16)
SEQ ID NO: 84	TAAATTTGAATTT	Eucalyptus Laccase (SEQ ID NO 17)

[0122] The interaction of bZIP proteins with vascular-specific or vascular-preferred promoters, for example, provides another example of how one of skill in the art can characterize regulatory elements. For example, the gene encoding glycine-rich protein, grp1.8, is expressed in a xylem-specific manner, as determined by histochemical staining of a GRP1.8-β-glucuronidase fusion protein. Keller *et al.*, *EMBO J.* **8**: 1309-14 (1989). A 5' deletion study of the grp1.8 promoter revealed a 20-base pair negative regulatory element that is essential for vascular-specific expression. Keller *et al.*, *Plant Cell* **3**: 1051-61. Similar xylem-specific regulatory elements that operate by negative tissue-specific regulation of a promoter have been identified in the bean pal2 and parsley 4CL-1. Leyva *et al.*, *Plant Cell* **4**: 263-71 (1992); Hauffe *et al.*, *Plant J.* **4**: 235-53 (1993), respectively. A transcription factor in xylem, VSF-1, specifically interacts with the 20-base pair regulatory element sequence of the grp1.8 gene. The interaction of VSF-1 and the regulatory element

was demonstrated by the formation of a high molecular weight complex between VSF-1 and the regulatory element, using an electrophoretic mobility shift assay, with the specificity of binding being demonstrated by the unlabeled DNA target competing for VSF-1 binding. Torres-Schumann *et al.*, *Plant J.* **9**: 283-96 (1996). The precise residues with which VSF-1 interacts were delineated further by measuring the sensitivity of the regulatory element to DNaseI. Briefly, the interaction of VSF-1 with the regulatory element prevents DNaseI from degrading certain residues that are contacted by the protein, by virtue of steric hindrance by the bound transcription factor. Typically, the DNaseI analysis also reveals residues that become hypersensitive to degradation when the transcription factor is bound. To verify that the putative regulatory element was xylem-specific, monomers or multimers of the putative regulatory region were fused upstream of a minimal CaMV 35S or minimal grp1.8 promoter. The putative regulatory region conferred xylem-specific expression on these promoters, as determined by the histochemical analysis using a fused β-glucuronidase gene operably linked to the modified promoter, stably transfected into tobacco. *See* Torres-Schumann (1996).

Functional variants or fragments of the promoters of the invention

[0123] Additional variants or fragments of the promoters of the invention are those with modifications interspersed throughout the sequence. Functional variants or fragments, as used herein, are nucleic acids that have a nucleic acid sequence at least about 70% identical to the reference nucleic acid, but still confer tissue-specific expression of coding products. The tissue-specificity or preference of the functional variant must be towards the same tissue as the reference nucleic acid. However, even if the functional variant is not as preferential or as specific as the reference nucleic acid, the variant is still considered a functional variant as used herein. In one embodiment, the sequence of the functional variant or fragment is at least about 75% identical to the reference nucleic acid. In other embodiments, the sequence of the functional variant or fragment is at least about 80%, 85%, 86%, 87%,. 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

[0124] Modifications that can produce functional variants may be made by sequential deletion of residues from the 5' end or the deletion of 5' UTR sequences from the 3' end. Alternatively, internal residues may be modified. Modifications that do not affect the function of the promoter regions most likely will be those that do not affect the binding of transcription factors. The modifications encompassed by the invention also include those that occur naturally in the form of allelic variants of the promoters of the invention. The variants of the current invention are tested for organ or tissue specific, or organ or tissue preferred activity using the methods described herein.

Methods of making the nucleic acids of the present invention

[0125] The nucleic acids of the invention can be obtained by using well-known synthetic techniques, standard recombinant methods, purification techniques, or combinations thereof. For example, the isolated polynucleotides of the present invention can be prepared by direct chemical synthesis using the solid phase phosphoramidite triester method (Beaucage *et al.*, *Tetra. Letts*. 22: 1859-1862 (1981)), an automated synthesizer (Van Devanter *et al.*, *Nucleic Acids Res.* 12: 6159-6168 (1984)), or the solid support method of U.S. Patent No. 4,458,066. Chemical synthesis generally produces a single stranded oligonucleotide, which can be converted into double stranded oligonucleotides by hybridization with a complementary sequence, or by polymerization, using the single strand as a template. Also, longer sequences may be obtained by the ligation of shorter sequences.

[0126] Alternatively, the nucleic acids of the present invention can be obtained by recombinant methods using mutually priming oligonucleotides. See *e.g.* Ausubel *et al.,* (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (John Wiley & Sons, Inc. 1990). Also, see Wosnick *et al.*, *Gene* 60: 115 (1987); and Ausubel *et al.* (eds.), SHORT PROTOCOLS IN MOLECULAR BIOLOGY, 3rd ed., (John Wiley & Sons, Inc. 1995). Established techniques using the polymerase chain reaction provide the ability to synthesize polynucleotides at least 2 kilobases in length. Adang *et al.*, *Plant Mol. Biol.* 21: 1131 (1993); Bambot *et al.*, *PCR Methods and Applications* 2: 266 (1993);

Dillon *et al.*, "Use of the Polymerase Chain Reaction for the Rapid Construction of Synthetic Genes," in METHODS IN MOLECULAR BIOLOGY, Vol. 15: PCR PROTOCOLS: CURRENT METHODS AND APPLICATIONS, White (ed.), pages 263-268, (Humana Press, Inc. 1993); Holowachuk *et al.*, *PCR Methods Appl.* 4: 299 (1995).

Methods of using the nucleic acids of the invention

[0127] The nucleic acids of the current invention are useful for altering characteristics of the plant. The nucleic acids may be operably linked to a gene of interest to increase the levels of a molecule found in the vascular tissue. Alternatively, the gene of interest may inhibit the formation of an ultimate end product, e.g. cellulose, thus the nucleic acids of the current invention can be used to decrease the levels of a predetermined molecule in vascular tissue.

[0128] One of the primary targets of such manipulated expression is the lignin biosynthetic pathway. For the reasons set forth above, there is considerable interest in regulating the amount of lignin in plants, either positively or negatively, which can be accomplished through expression of gene products that impact this pathway. For example, manipulation of the number of copies of CAD and COMT modifies lignin content, as described in U.S. Pat. No. 5,451,514 and WO 94/23044. Furthermore, antisense expression of sequences encoding CAD in poplar or pine leads to a modified lignin composition. Grand *et al.*, *Planta (Berl.)* 163: 232-37 (1985); Baucher *et al.*, *Plant Physiol.* 112: 1479 (1996), respectively.

[0129] Other characteristics which may be important to alter include sugars. Increasing the levels of enzymes responsible for sugar production, for example, in phloem tissue would increase the sugar content in the plants. Enzymes which may increase or decrease the levels of sugar production include, but are not limited to β-glucosidases, 1-3 β glucanase, 6-phospho-fructo-1-kinase, sucrose synthase, UDP-glucose-pyrophosphorylase, hexokinase, phosphoglucomutase, sucrose transporters, and invertases.

[0130] Additional characteristics include, but are not limited to, increasing insecticidal, antiviral or antibacterial proteins present in the leaves of the plant. Such proteins include, but are not limited to cecropins, thionins, defensin, lipid transfer proteins, hevein-like peptides, and GASA peptides. Still other characteristics include drought tolerance, cold tolerance, peroxidase activity, salt tolerance, and nitrogen transport.

[0131] The present invention, thus generally described, will be understood more readily by reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention.

EXAMPLES

Example 1: Isolation of Vascular-Specific Promoters

[0132] *Pinus radiata* and *Eucalyptus grandis* cDNA libraries were constructed and screened as follows. mRNA was extracted from plant tissue using the protocol of Chang *et al.*, *Plant Molecular Biology Reporter* 11:113-116 (1993) with minor modifications. Specifically, samples were dissolved in CPC-RNAXB (100mM Tris-Cl, pH8.0; 25mM EDTA; 2.0M NaCl; 2% CTAB; 2% PVP and 0.05% Spermidine*3 HCl) and extracted with chloroform:isoamyl alcohol 24:1.

[0133] RNA was precipitated with ethanol and purified using MessageMaker kit (Life Technologies). A cDNA expression library was constructed from purified mRNA by reverse transcriptase (Loopstra *et al.*, *Plant Mol Biol.* 27:277-291 (1995)) synthesis followed by insertion of the resulting cDNA clones in Lambda ZAP using a ZAP Express cDNA Synthesis Kit (Stratagene), according to the manufacturer's protocol. The resulting cDNAs were packaged using a Gigapack II Packaging Extract (Stratagene) employing 1 μl of sample DNA from the 5 μl ligation mix. Mass excision of the library was done using XL1-Blue MRF' cells and XLOLR cells (Stratagene) with ExAssist helper phage (Stratagene). The excised phagemids were diluted with NZY Broth (Gibco BRL, Gaithersburg, MD) and plated out onto LB-

kanamycin agar plates containing 5-bromo-4-chloro-3-indolyl-beta-D-galactoside (X-gal) and isopropylthio-beta-galactoside (IPTG).

[0134] Colonies were picked and DNA prepared using standard miniprep technology. Colonies containing a correct insert were cultured in NZY Broth with kanamycin and cDNA was purified by means of alkaline lysis and polyethylene glycol (PEG) precipitation. Agarose gel at 1% was used to screen sequencing templates for chromosomal contamination. Dye terminator chemistry was set up using Big Dye Chemistry (Applied Biosystems), according to the manufacturer's protocol.

[0135] The DNA sequence of positive clones were obtained using a 3700 Capillary Machine (Applied Biosystems) or a Prism 377 sequencer Perkin Elmer/Applied Biosystems Division. cDNA clones were sequenced first from the 5' end, and in some cases, also from the 3' end. For some clones internal sequence was obtained using subcloned fragments. Subcloning was performed using standard procedures of restriction mapping and subcloning into pBluescript II SK+ vector.

[0136] Plant EST sequences homologous to a selected gene were identified from either a *Pinus radiata* or *Eucalyptus grandis* cDNA expression library and are displayed in Table 2.

Table 2

SEQ ID NO	Gene Homology	Species	BLASTX Hit	E Value	Identity
1	Glucosyl Transferase	*E. grandis*	Glucosyl Transferase	e-130	214/421 (50%)
2	Ted 2 (970 bp)	*E. grandis*	NADP-Dependent Oxidoreductase Ted2	e-160	276/324 (85%)
3	Cyclin B (447 bp)	*E. grandis*	Putative G2/Mitotic-Specific Cyclin 1 (B-like Cyclin)	e-122	242/360 (67%)
4	Cyclin B (883 bp)	*E. grandis*	Putative G2/Mitotic-Specific Cyclin 1 (B-like Cyclin)	e-122	242/360 (67%)
5	Microtubule associated	*E. grandis*	AT2G45170 Putative Microtubule-Associated Protein	2.00E-53	100/117 (85%)
6	CAP Adenyl Cyclase Associated	*E. grandis*	Adenyl Cyclase Associated Protein	0.0	382/476 (80%)

7	Ferulate-5-Hydroxylase	*E. grandis*	Ferulate-5-Hydroxylase	0.0	402/517 (77%)
8	Cellulose synthase	*E. grandis*	Cellulose Synthase	0.0	845/978 (86%)
9	LIM promoter	*P. radiata*	LIM Domain Protein PLIM1	3.00E-78	126/175 (72%)
10	Pectate Lyase	*P. radiata*	Pectate Lyase	0.0	298/379 (78%)
11	Expansin	*P. radiata*	Alpha-Expansin	e-122	197/240 (82%)
12	ACC Oxidase 1 1-Aminocyclopropane-1-carboxylate cxidase	*P. radiata*	ACC Oxidase	9.00E-99	187/321 (58%)
13	4-Coumarate CoA Ligase 3(4CL3) (contains 5'-end 40 bp insert)	*P. radiata*	4-Coumarate-CoA Ligase (EC 6.2.1.12)	0.0	392/533 (73%)
14	4CL1 or 4CL2 (650 bp)	*P. radiata*	4-Coumarate-CoA Ligase (EC 6.2.1.12)	1.00E-89	168/175 (96%)
15	4CL1 or 4CL2 (1650 bp)	*P. radiata*	4-Coumarate-CoA Ligase (EC 6.2.1.12)	3.00E-89	168/175 (96%)
16	Dirigent Protein	*P. radiata*	Dirigent Protein, Putative (Hypothetical	5.00E-41	77/150 (51%)

			20.6 KDa Protein)		
17	Laccase (898 bp)	*E. grandis*	Laccase Precursor (EC 1.10.3.2)	e-153	263/318 (82%)
18	Laccase (500 bp)	*E. grandis*	Laccase Precursor (EC 1.10.3.2)	e-153	263/318 (82%)
19	Unknown protein Euc E11912 (600 bp)	*E. grandis*	AT4G27438	8.00E-65	122/172 (70%)
20	Cellulose-synthase like	*P. radiata*	Putative Glucosyl Transferase	0.0	487/690 (70%)
21	4CL3 (without 5'-end 40 bp insert)	*P. radiata*	4-Coumarate-CoA Ligase (EC 6.2.1.12)	0.0	392/533 (73%)

[0137] Polynucleotide or polypeptide sequences can be aligned with other polynucleotide and/or polypeptide sequences and the degree of shared identical sequence can be determined using computer algorithms that are publicly available. The BLAST family of algorithms, including BLASTN, BLASTP, and BLASTX, is described in the publication of Altschul *et al.*, "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs," Nucleic Acids Res. 25:3389-3402, 1997. The BLASTN algorithm Version 2.0.11 [Jan-20-2000] set to the default parameters described in the documentation and distributed with the algorithm, are preferred for use in the determination of polynucleotide variants according to the present invention.

[0138] BLASTX searches were performed using SWISSPROT- TREMBLE Sequences [July-9-2002] and the searches were performed on November-15-2002. The following running parameters are preferred for the determination of alignments and similarities using BLASTX that contribute to the E values and percentage identity of polypeptide sequences: blastall –p blastx –d swissprotdb –e 10 –G 0 –E 0 -FF –v 30 -b 30 -i queryseq -o results; the parameters are: -p Program Name [String]; -d Database [String]; -e Expectation value (E) [Real]; -G Cost to open a gap (zero invokes default behavior) [Integer]; -E Cost to extend a gap (zero invokes default behavior) [Integer]; -F Filter Query Sequence [String]; -v Number of one-line descriptions (v) [Integer]; -b Number of alignments to show (b) [Integer]; -I Query File [File In]; -o BLAST report Output File [File Out] Optional.

[0139] The promoters were cloned using a "Genome Walker" kit (Clontech, Palo Alto, CA). This is a PCR-based method, which requires four PCR primers to be constructed, two of which must be gene-specific. The gene specific primers are designed generally within the 5' UTR of the gene. The fragment is amplified and then cloned into a T-tailed vector in front of the GUS reporter gene.

Example 2: Methodology to Determine the Tissue Specificity of a Promoter

[0140] Following the identification and cloning of a promoter by the procedure outlined above in Example 1, the promoter is operably linked with a reporter gene to determine those tissue types in which the promoter is active. To this end, a construct containing an inventive promoter is transformed into *Agrobacterium tumefaciens* by electroporation. Briefly, 40 μl of diluted AgL-1 competent cells are placed on ice and are contacted with about 10 ng of pART27 vector containing the promoter sequence. Electroporation is conducted under the following parameters:

Resistance = 129 ohm

Charging voltage = 1.44 kV

Field strength = 14.4 kV/cm

Pulse duration = 5.0 ms

[0141] Following electroporation, 400 μl of YEP liquid media is added and the cells are allowed to recover for one hour at room temperature. Cells then are centrifuged at 6000 rpm for 3 min and are resuspended in ~50 μl YEP. Cell samples are spread over the surface of a YEP Kan50 / Rif50 plate, sealed with parafilm, and incubated at 29°C for 2 days for colony growth.

[0142] Wild type *Arabidopsis thaliana* cv. 'Columbia-0' plants are then transformed with *Agrobacterium* containing constructs of interest by floral dip infiltration. Briefly, *Agrobacterium* cultures are centrifuged at ~8600 rcf for 10 min at 20°C and are resuspended to an optical density of ~0.7 – 0.8. Plants are dipped into an infiltration solution containing the *Agrobacterium* for 5 sec. Plants are drained of excess solution and placed under grow lights in ambient conditions. After 24 hrs, the plants are misted and maintained for seed production. T_1 seeds are surface sterilized in 5% commercial bleach solution and plated on MS media containing Kanamycin (50 mg/l) and Timentin (250 mg/l) to select for putative transformants.

[0143] *N. benthamiana* plants are transformed with constructs of interest by *Agrobacterium*-mediated leaf tissue transformation (Burow *et al.*, *Plant Mol. Biol. Rep.* 8:124-139, 1990).

[0144] Successfully transformed plants are then assayed for the expression of the operably linked reporter gene. Leaf, stem, root and floral regions are immersed in a staining solution (50 mM $NaPO_4$, pH 7.2, 0.5% Triton X-100, 1 mM X-Glucuronide, cycloheximide salt (Ducheffa). A vacuum is applied twice for 5 min to infiltrate the tissue with the staining solution. The tissue is then left shaking overnight at 37°C for color development. Tissues are checked at three or four time-points to check stain development, and if samples show early development, a piece of tissue is destained in 70% ethanol. This tissue is then examined for GUS expression using a light microscope and photographed.

Example 3: Isolation and Culture of *Zinnia elegans* Mesophyll Cells in Tracheary Element (TE) Inducing (FKH) and Non-Inducing (FK) Medium.

[0145] Primary and secondary pair leaves from *Zinnia* seedlings were harvested from 8 punnets. Leaves were sterilized in 500 ml of 0.175% sodium hypochlorite solution for 10 minutes. Leaves were then rinsed twice in 500 ml of sterile water. Using 20-30 leaves at a time, leaves were ground in 25-30 ml of FK medium with a mortar and pestle. The cells were filtered through a 40 μm nylon mesh and a total of 90 ml of mesophyll cells were obtained in this fashion. The cells were centrifuged at 200 x g for 2 minutes at 20° C and the resultant pellet was washed once more with an equal volume of FK medium. Upon completion of the second wash, the pellet was split into two equal halves, wherein one half was washed with 45 ml of FK medium and the other with 45 ml of FKH medium. The pellets were resuspended in 60 ml of FK medium and 60 ml of FKH medium, respectively. They were cultured in the dark in two 6-well plates on the rotary shaker set at 120 rpm.

Example 4: Isolation of *Zinnia elegans* Protoplasts from Leaves or Mesophyll Cells Cultured Overnight to Three Days in FK Medium and FKH Medium.

[0146] Sterile *Zinnia elegans* primary leaves (6-8 in number) were cut into 1 mm slivers and placed in 15 ml of cell wall digesting enzyme mix (1% Cellulase Onozuka R-10 and 0.2% pectolyase Y23 in Protoplast isolation buffer). Mesophyll cells cultured in FK medium (40 ml) or FKH medium (40 ml) were pelleted by centrifuging at 200 x g for 2 minutes at 20° C. Each pellet was resuspended in 20 ml of sterile protoplast isolation buffer containing 200 mg Cellulase Onozuka R-10 and 40 mg Pectolyase Y23. The protoplasts were isolated by incubating the cell suspensions in CellStar culture plates for 2-4 hours on a rotary shaker set at ~70 rpm at 23° C. The protoplasts were pelleted by centrifuging the contents of the plates at 200 x g for 2 minutes. Each pellet was then resuspended in 20 ml of 24% sucrose solution.

Fukuda and Komamine Medium Stock Solutions

Stock A (10X)	Stock B (400X)	Stock C (400X)	Stock D (400X)	Stock E (400X)
20,200 mg KNO_3	2,500 mg $MnSO_4.4H_2O$	3,700 mg Na_2EDTA	200 mg Glycine	50 mg Folic Acid
540 mg NH_4Cl	1,000 mg H_3BO_3	2,800 mg $FeSO_4.7H_20$	10, 000 mg myo-Inositol	250 ml Milli-Q water
2,470 mg $MgSO_4.7H_20$	1,000 mg $ZnSO_4.7H_2O$	250 ml Milli-Q water	500 mg Nicotinic acid	
1,470 mg $CaCl_2.2H20$	25 mg $Na_2MoO_4.2H_2O$		50 mg pyridoxine hydrochloride	
680 mg KH_2PO_4	2.5 mg $CuSO_4.5H_20$		5 mg thiamine hydrochloride	
1,000ml Milli-Q water	250 ml Milli-Q water		1,000 ml Milli-Q water	

For 1 Litre Solution	FK Medium	FKH Medium
Stock A	100 ml	100 ml
Stock B	2.5 ml	2.5 ml
Stock C	2.5 ml	2.5 ml
Stock D	2.5 ml	2.5 ml
Stock E	2.5 ml	2.5 ml
Sucrose	10,000 mg	10,000 mg
d-(-) Mannitol	36,000 mg	36,000 mg
1-Naphthaleneacetic acid (NAA		0.1 mg
6-Benzyladenine (BA)		0.2 mg
Milli-Q water	up to1,000 ml	up to1,000 ml

Example 5: Transfection of *Zinnia elegans* Protoplasts

[0147] *Zinnia* protoplasts suspended in 24% sucrose solution were overlaid with 1 ml W5 solution (150 mM NaCl, 125mM $CaCl_2.2H_2O$, 5 mM KCl, 5 mM sucrose, pH 5.6 – 6) and centrifuged at 70 x g for 10 minutes at 20° C with brakes off. Floating protoplasts were harvested, resuspended in 10 ml W5 solution, and pelleted by centrifuging at 70 x g for 10 minutes at 20° C. Protoplast pellets were resuspended in

MaMg medium (450mM mannitol, 15mM $MgCl_2$, 0.1% MES, pH 5.6) at a density ~5 x 10^6 protoplasts/ml and aliquoted into individual 15 ml tubes (300 μl: 1.5 x 10^6 protoplasts). For each construct, 5 μg DNA and 50μg Salmon Testes DNA was added to the protoplast suspension, mixed, and incubated for 5 minutes at 20° C. Three hundered μl 40% PEG solution (40% PEG 3340, 100 mM $Ca(NO_3)_2.4H_2O$, 0.45 M mannitol, pH 9.0) was added to each protoplast aliquot, mixed, and incubated for 20 minutes at 20° C. Five ml of K3/0.4M sucrose (for 1 L solution: 4.3 g Murashige and Skoog plant salts, 100 mg Myo-inositol, 250 mg xylose, 10 mg Thiamin-HCl, 1 mg Nicotinic acid, 1 mg Pyridoxin-HCl, 1 mg NAA, 0.2 mg kinetin, 137 g sucrose, pH 5.6 adjust with KOH) was added to each aliquot of leaf-derived transfected protoplasts or transfected protoplasts from mesophyll cells cultured in FK medium and mixed. Similarly, 5 ml of K3/0.4M sucrose+0.1ppm NAA+0.2ppm BA was added to each aliquot of transfected protoplasts from mesophyll cells cultured in FKH medium and mixed. The transfected protoplast suspensions were incubated overnight at 23° C in the dark.

Example 6: Harvesting of Transfected *Zinnia elegans* Protoplasts and Reporter Gene Analysis

[0148] Transfected *Zinnia* protoplast suspensions, prepared as described in Example 5, were individually harvested by adding 9.5 ml of W5 solution, mixing the contents of each tube, and centrifuging at 70 x g for 10 minutes at 20° C. The bulk of the supernatant was decanted and the protoplast volume was brought to 900 μl. For each 900 μL sample, 300 μL of protoplasts were aliquoted into 5 ml polystyrene round-bottom tubes, resuspended in 500 μl W5 medium, and set aside for analysis of fluorescent reporter gene expression and cell viability. The remaining 600 μL of protoplasts and solution were transferred into individual microtubes and pelleted by centrifugation at 420 x g for 2 minutes at 20° C. The protoplast pellet was assayed for GUS reporter gene expression as described by Jefferson, R.A.,1987, *Plant Mol. Biol. Rep.* 5, 387. GUS (MUG) assays were performed using a Wallac (Turku, Finland)

Victor [2] 1420 Multilabel Counter. Umbelliferone was detected using a 355 nm excitation filter and a 460 nm emission filter for 1 second.

Example 7: *In Planta* Expression Data

[0149] Three weeks post-transfer to soil, transformed *Arabidopsis* and *N. benthamiana* tissues are analyzed for GUS reporter gene expression. To assay GUS expression, leaf, root, and floral materials are immersed in the GUS solution as described in Example 2. A vacuum is applied twice for 5 minutes to infiltrate the tissue with the staining solution and the tissue is then incubated overnight in a shaker at 37°C for color development. Following overnight incubation, the tissue samples are then destained in 70% ethanol and examined under a light microscope for GUS expression. Table 3 contains the percentage of transformed *Arabidopsis* and *N. benthamiana* plants expressing GUS.

[0150] Three months post-soil transfer, tissues from *N. benthamiana* T_1 plants are embedded into paraplast, sectioned with a microtome, and analyzed with a light microscope for GUS expression. The GUS localization and microtome results, as shown in Table 3, demonstrate that the disclosed isolated nucleotide sequences confer reporter gene expression preferentially in vascular cambium, xylem, and/or phloem tissues.

Table 3: *In planta* GUS Vascular Expression

SEQ ID NO	Size (bp)	No Plants GUS +	No Plants Tested	% GUS Expression	GUS Vascular Localization	Microtome Results
1	168	2	12	16.67	Stem material	
2	934	7	12	58.33		Cambial cells, early xylem
3	408	11	12	91.67	Stem, developing lateral roots	Cambium, xylem
4	847	11	12	91.67	cambium	Cambium initials, lateral roots
5	286	8	12	66.67	Phloem, pith, Phloem parenchyma	
6	216	4	12	33.33	xylem	

7	473	7	10	70.00	Not specified	
8	519	8	10	80.00	Not specified	
9	1607	11	12	91.67	Leaf veins, roots	
10	1163	2	12	16.67	Stem pith, primary xylem	
11	881	11	12	91.67	Stem, trichomes	Primary xylem cells
12	638	5	5	100.00	Stem, leaf veins, root tip	
13	900	8	12	66.67	Primary xylem, cortex, pith, anthers, tip of style	
14	603	10	10	100.00	Stem, leaf veins	
15	1631	7	12	58.33	Roots, leaf veins, stem	Primary xylem, cambial cells, xylem ray cells
16	786	12	12	100.00	Parenchyma, pith	Parenchyma, pith, xylem
17	898	5	10	50.00	Phloem, leaf veins, cortex-parenchyma cells/phloem cells, and epidermal cells	
18	563	2	7	28.57	Primary xylem, parenchyma cells	
19	524	2	12	16.67	Late developing xylem, roots	
20	638	5	12	41.67	Inner pith, parenchyma cells, xylem	
21	862					
22	691				Stem pith	
23	970	7	12	58.33	Vascular tissue	Cambial initials, early xylem
24	447	11	12	91.67	Stem, Vascular tissue, lateral roots	Differentiating Cambial initials, xylem,
25	883	11	12	91.67	Vascular tissue	Cambial initials, xylem, lateral roots,
26	500	7	10	70.00	Vascular tissue	
27	650	8	10	80.00	Vascular tissue	
28	252	4	12	33.33	Vascular tissue	Xylem, branch points
29	322	8	12	66.67	Vascular tissue	Pith, cortex, phloem
30	204	2	12	16.67	Vascular, stem material	
31	784	6	8	75.00	Vascular tissue	
32	1161				Vascular tissue	Phloem, xylem, cambium
33	500	2	7	28.29	Vascular tissue	Xylem, parenchyma
34	524	5	12	41.67	Vascular tissue	Early, developing xylem,

						metaxylem
35	1766	12	12	100.00	Vascular tissue	Xylem, cambial cells, secondary xylem
36	650	12	12	100.00	Vascular tissue	Phloem, xylem
37	894	9	9	100.00	Vascular tissue	Primary xylem
38	583	3	6	50.00	Vascular Tissue	Primary xylem
39	898	11	11	100.00	Vascular Tissue	Xylem
40	611	6	7	85.86	Vascular Tissue	Inner pith cells
41	537	7	10	70.00	Vascular Tissue	Primary Xylem, phloem
42	362	11	11	100.00	Vascular Tissue	Xylem
43	810	8	9	88.89	Branch, base tissue	Xylem
44	335	10	12	83.33	Vascular	Xylem
45	476	8	9	88.89	Stem, vascular	
46	536	8	11	72.73	vascular	Xylem vessels,
47	716	6	7	85.86	vascular	Xylem, cambial, ray cells
48	1643	11	12	91.67	Vascular, stem material	xylem
49	917	11	12	91.67	Vascular, stem material	Primary xylem
50	900	8	12	66.67	Vascular material	Primary xylem,
51	650	10	10	100.00	Vascular material, stem section	Xylem, lignified xylem
52	1650	12	12	100.00	Vascular material	Xylem vessels
53	1200	2	12	16.67	Stem material, pith	Phloem, xylem
54	674	5	5	100.00	Stem, veins	
55	822	12	12	100.00	Parenchyma, stem	Xylem, cortex, thickened xylem cells
56	1300	2	12	16.67	Vascular material	
57	674	5	12	41.67	Vascular material	Xylem, pith, parenchyma cells
58	1350	9	12	75.00	Vascular material	Xylem rays, xylem
59	700	2	7	28.57	Stem sections	Xylem ring, cortex

[0151] *N. benthamiana* tissue samples are placed in a vial containing fixative solution (100% Ethanol, Glacial acetic acid, 37% Formaldehyde, mQH_20) and a vacuum is applied twice for 15 minutes. The samples are incubated in the fixative solution for two hours to ensure tissue infiltration. Following tissue infiltration, a vacuum is reapplied for 15 minutes and the tissues are placed at 4^oC overnight.

[0152] Following overnight incubation, the tissue samples are dehydrated through a series of ethanol incubations, wherein all incubations occur at room temperature. The

overnight fixative solution is removed from the vial containing the tissue samples and replaced with 50% ethanol. After 30 minutes, the 50% ethanol is decanted and the tissues are incubated in a fresh aliquot of 50% ethanol. After 30 minutes in 50% ethanol, the solution is removed and replaced with 60% ethanol. Following 30 minute incubation, 60% ethanol is replaced with 70% ethanol. The 70% ethanol is decanted after 30 minutes and replenished with 85% ethanol. Following 30 minute incubation, the 85% ethanol is removed and the samples are incubated overnight in 95% ethanol.

[0153] Following tissue dehydration, the tissues are incubated at room temperature in a series of xylene solutions. The overnight 95% ethanol solution is removed, and the tissues are incubated in 100% ethanol for 30 minutes. After 30 minutes, 100% ethanol is removed and the tissues are suspended in 25% Xylene:75% Ethanol. Following 30 minute incubation, the solution is replaced with 50% Xylene:50% Ethanol. The solution is then decanted and replenished with 75% Xylene:25% Ethanol. After 30 minutes, the tissues are thrice incubated in 100% Xylene for 60 minutes. The tissues are then overnight incubated in a vial containing xylene and 20 paraplast chips.

[0154] To infuse the paraffin, the vials are placed in a 42°C hybridization oven until the paraplast chips dissolve. Throughout the course of 8 hours, a total of 60-80 paraffin chips are added to the vial and allowed to dissolve. The samples are left overnight in a 62 °C hybridization oven. Over the course of the next two days, the paraplast is changed four times, at 12 hour intervals. To embed the tissues, the liquid paraffin is poured into the cassette and the tissues are placed in the proper orientation. The cassette is then placed at 4°C overnight to allow the paraffin to harden.

[0155] As illustrated in Table 3, the disclosed isolated nucleotide sequences confer GUS reporter gene expression preferentially in vascular cambium, xylem, and/or phloem tissues. Of particular interest, SEQ ID NO 21 and SEQ ID NO 13 share substantial sequence homology, except SEQ ID NO 13 contains a 40-bp fragment insertion at its 5'-end **(AAATATAACATAATCTAACTATTGATGTACATT ATTCGCC).** This 40-bp fragment (SEQ ID NO: 85) is present as an imperfect

repeat in SEQ ID NO 13, yet is absent in SEQ ID NO 21. Interestingly, in the presence of the 40-bp fragment (*e.g.* SEQ ID NO 13), GUS expression is conferred, while in the absence of the fragment (*e.g.* SEQ ID NO 21), there is no GUS expression. This data is significant because it suggests that the disclosed 40-bp fragment (SEQ ID NO: 85) may constitute an element necessary and sufficient for vascular-preferred gene expression.

Example 8: Methods of Using a Vascular-Specific Promoter

[0156] Once a promoter having an appropriate tissue-specific and developmental pattern of expression is found, this promoter can be used to regulate a desired characteristic in a transgenic plant. In one embodiment, a xylem-specific promoter is used to regulate the composition and content of lignin in a plant. In this example, a xylem-specific promoter of the invention is operably linked to a gene encoding an RNA interference (RNAi) molecule corresponding to a portion of the coding region of *cinnamyl alcohol dehydrogenase* (*CAD*). CAD catalyzes the last step of lignin monomer synthesis and has provided a target for successful antisense-mediated down-regulation of lignin in transgenic plants using other promoters. See Yahiaoui *et al.*, *Phytochemistry* 49: 295-306 (1998) and references cited therein. Expression of an RNAi molecule corresponding to a portion of CAD results in a decrease in the enzyme activity and a corresponding increase in the proportion of cinnamyl aldehydes in the lignin of the transgenic plants. CAD activity in transgenic plants is assayed by the method of Wyrambik *et al.*, *Eur. J. Biochem.* 59: 9-15 (1975) as adapted by Baucher *et al.*, *Plant Physiol.* 112: 1479-90 (1996). Lignin content and composition are measured as set forth by Baucher (1996). Down-regulation of CAD results in a color change in the wood and an increase in the proportion of more easily extracted cinnamyl aldehydes, which is commercially important for plants used in the pulp industry. *Id.* For example, poplars with decreased CAD activity display red coloration, and the lignin is more easily extractable, which improves the properties of the poplar trees for application in the paper industry. See Baucher *et al.* (1996).

[0157] In another example, Eucalyptus and pine are transformed. Eucalyptus and pine provide useful model systems for studying the effect of gene regulation on lignin content and composition because of its the high lignin content. Additionally, Eucalyptus and Pine exemplify plants in which the modulation of lignin content or composition can provide useful properties with respect to the paper or lumber industries.

[0158] To construct these transgenic plants, a fragment of CAD cDNA is operably linked in proper orientation to a xylem-specific promoter of the invention and a nopaline synthase 3' terminator. The CAD cDNA can be prepared, for example, by reversing the orientation of the gene sequence with respect to its promoter. Transcription of the CAD cDNA in the plant cell will generate an intracellular RNA transcript that is "antisense" with respect to the CAD gene. The entire construct is inserted as a restriction fragment into the binary vector pBI101.1 (Clontech, Palo Alto, CA). Vectors are electroporated into *A. tumefaciens* strain LBA4404 or C58pMP90, for eucalyptus or pine transformations, respectively. See generally, No *et al.*, *Plant Science* 160: 77-86 (2000). Kanamycin resistant transformants are tested for CAD activity, transgene copy number is determined by Southern analysis, and mRNA expression of CAD is determined as described in Yahiaoui (1998). Suitable transformants then are rooted and transferred to a greenhouse.

[0159] Example 9: *In silico* Expression

[0160] In silico gene expression can be used to determine the membership of the consensi EST libraries. For each library, a consensus is determined from the number of ESTs in any tissue class divided by the total number of ESTs in a class multiplied by 1000. These values provide a normalized value that is not biased by the extent of sequencing from a library. Several libraries were sampled for a consensus value, including Cambium, Xylem, Phloem, Vegetative Bud, Root, Reproductive tissues, Leaf, Stem, and Fruit libraries. As shown below in Tables 4-5, a number of the inventive promoter sequences exhibit vascular-preferred expression and thus are likely to be involved in wood-related developmental processes.

Table 4: *Eucalyptus grandis* Expression Profile

SEQ ID NO	Bud (Veg)	Cambium	Fruit	Leaf	Phloem	Reproductive	Root	Stem	Xylem
24	0.42	1.99	0.08						2.33
27		0.35	0.54		0.17		0.12	0.95	7.71
31	0.59			0.17					
33	0.08	3.69	0.85	1.56	0.28		0.08	0.95	15.68
36				0.83					
37		1.31	0.15		0.17	4.01	0.32		1.23
59									
60		11.98	0.15	3.35	0.17		0.08	0.95	47.12
61	0.25	4.69	0.15	0.51	0.83	6.69	0.08	0.24	3.78
62				0.35			0.35		0.47

Table 5: *Pinus radiata* Expression Profile

SEQ ID NO	Bud (Veg)	Cambium	Fruit	Leaf	Phloem	Reproductive	Root	Stem	Xylem
24	0.42	1.99	0.08						2.33
27		0.35	0.54		0.17		0.12	0.95	7.71
31	0.59			0.17					
33	0.08	3.69	0.85	1.56	0.28		0.08	0.95	15.68
36				0.83					
37		1.31	0.15		0.17	4.01	0.32		1.23
59									
60		11.98	0.15	3.35	0.17		0.08	0.95	47.12
61	0.25	4.69	0.15	0.51	0.83	6.69	0.08	0.24	3.78
62				0.35			0.35		0.47

TABLE 6

SEQ ID NO	Gene Homology	Sequence
1	*E. grandis* Glucosyl transferase	AAAACCACAAATGGCCGCGGGACGTCACAATTTTTTTTCCTTCTAGAAGCTCTATAGTCAAAGCTGATCTATAAATTTT TGGGAACCACAACCACCATGTCTCGCCACCTTCGCTCGAACCTTATCACCACCACCGCCCTTGAGCCCTCCTCCATCAAC TCTTCTTC
2	*E. grandis* Ted (2)	AAAACAATGTAGCTTCTCTGTGTTATGAAAACTAACAAAAGGGCACATCTATTTCTCCATGACCATTATATTCGGAGGAG CATGGTCAAACTTAAATCAAAATTTATTATCCATAACTTACAAATTTCCAATTTAGCTAAACTCAAATCCCAAAGTATAG CAATTCTGTTAAAATTTTATCATCCGATAGTATAGAGCAATATTTTATAATACTTACATTGCTCAGCTCAATTACAAATTC TATTTGTCCACAAATTCAAACATTTTAATGATGCATTCCACATAAAACCAATGGTTTGAGACACCTTTTCAAAAAAAAGA AAAAAATACACTAGCATTGCTTAGACAAGTTAATCAATGAAAAATAACTTTATCTTGTTTTTAATTAAGGATGAAAAGG AGTTACAAACGCTTGTTTCAAGATAAATATTTTTCAAATCTTTAATATTACAAGAAATAAACGGACCTTCTTATCAACCA AAAAAATGTAACATAAAAGGAACTTACCAATTTGATTGGACTCATTTATTGATTTTTGGAAAAATGTCGCAAATTTTCGT TGAGTTTTAGCTCCATGTACAATTTAGTCATTGAACTTTTAATTTATTCAATATAATTCATGAACTTTCTATACATATTTA GTCCATATAAAAATTAAGGGACCAAATTGAGTATTCACCAAAATTTTAGGGAAAATATTGAATAAATAAAAAGTTCTTG GACCAAATTTCATATTGAAATAAAATTCATGGACAAATCATTATTCCTTGATTAAACTTTTTTATGTAGACACCCGTAAA TACAACCTGCCAAGGTTTGTTTGCAAGGCGTTTGCAAGGCGTTTGCACTTAAGCGGGACGGAGGCGTCACCAGTCAATG GGCATGTCCAGTGGCTTCCCCGGCTTGCGAATAGGATGCTTCCTGAATCATCTCC
3	*E. grandis* Cyclin B	AAAGTTTCTCTGTAGAGAGAGGGAGGGAGATATATCTGCGGTTTGCGTCTCTATTTCGCTTGTGCAGTTTTACTACTCCC CAAACACACACACACTCTCTCTGTTTCTCTCCTTTTTCCCCAAATCAGAAGAAGAAACGACAGTGTAGTAGTGCAGTTTC ACTACACCGTCTATACTAAGGGTAATCGTTTTTTTGAAAGCACATGCATATAGCCGTTGGAAAGGGGAGGGCACCGAGA TCGAATCGGATGGCTGATCCTCACTAGCCGTTAGAGAGAGAGAGAGAGAGGGAGGGATAATCATGTGCGGACATATAT CCGCAATTTGCGTCTCTATTTCGCTTGTGCAGTTTCACTACTCCCCACACACACTCTCTCTCTCTCTCTCCTTTTCCCCC AAATCAGAA

4	*E. grandis* Cyclin B	CCTTGTATTTCCCCAACATTAAATGAAAGCCTACATCCAAAAACGTGGACCCGGCATTAAAGAAAAACCCCATCATCTC ATCCCATCCTTTATTTCAACCCTAAAGTGAAATTAAGATATAAGACGAAACCACCCCCAACCCCCCAAAAAAAAAATAT TAAGGGAATTCGTTTTTTTGAAAGCACATGCGGAGGTAGCTGTTGGAAAGGGGCCTCTACGTTCGGAAGGAATGCGACC ATTCCATCGAGATCAAATCGAACTACTGATGCTCACTAGCTGTTGCGTTTAAACCTTCTTTGTAAAGCGATAAGGGAATT CGTTATTTTGAAAGCACATGCGGAGGTAGCCATTGGAAAGGGGCCTCTACGTTCGGAAGGAACACGACCGTTCCACCGA GATCGAATCGGACCGTTGATGCTCACTAGCCATTGTGTTTAAAGTTTCTCTGTAGAGAGAGGGAGGGAGATATATCTGC GGTTTGCGTCTCTATTTCGCTTGTGCAGTTTTACTACTCCCCAAACACACACACACTCTCTCTGTTTCTCTCCTTTTTCCCC CAAATCAGAAGAAGAAACGACAGTGTAGTAGTGCAGTTTCACCACACCGTCTATACTAAGGGTAATCGTTTTTTTGAAA GCACATGCATATAGCCGTTGGAAAGGGGAGGGCACCGAGATCGAATCGGACGGCTGATCCTCACTAGCCGTTAGAGAG AGAGAGAGAGAGGGAGGGATAATCATGTGCGGACATATATCCGCAATTTGCGTCTCTATTTCGCTTGTGCAGTTTCACTA CTCCCCACACACACTCTCTCTCTCTCTCTCTCTCCTTTTCCCCCAAATCAGAA
5	*E. grandis* Microtubule- Associated Protein	AAATTATGCAATTTCTTAATCAGGCCTAGCTAGAAACAAGGGCAAGGAAAGCCCCCGACGGGCTCTTATCTGCTGACGT GGCACGCCGTGGGTGGGCCCCCCCGGGTCTTCCTTCGACGAAACCTCATCGTAGACAATCAAATCCTCCTCTCGATCATT ATTGCAAAGCCAACACCCAGCATTGAATCGATCCCCACCTTCTCCTCCTCCTCCTCTTGATCCTTTTTGTCCCGATGATGA TGGGTATCTGATCAGCCGATTCAATCCCATCGTCTCCTTCCTTCTC
6	*E. grandis* CAP adenyl cyclase	GTGCGGACACGTGTCCCCTTATCCCGCCCAAGACCGCGCAAAACCTGAAAATCCTCACTATTCCCTCACTTTCGGCGAAT TCGAAACAGCGCATAAAGGAACACGGAAAGAACATTCTCTACCCCAAGACGACGACGACGACGACGACGACGACGCCG CGCCTTATATAAACCATCGCCACTCCTGGCCATTCCCTTCTTTCTCCCCAGATCCAAT
7	*E. grandis* Ferulate-5- Hydroxylase	AAAGATAAAAATAGTGTGGAAAATAGATTTGAGAAGTGTTCATATATTTCGATTTATCATAGCAAAGATTTTATCGACCT ATTTTAGGCTTTATAGTGTGACTATTTAAGATAACGAATATTAATCGAGAGATGCACAATTAATAAGAGATATTCTCACG ATCTTGAGATATATAGAAACCGACAGAAAATATATTGATTATCTCTAATATAGAATAATATTCTAGAGAAGTATTGTAAT TGTGACCACCAACTAAAATGGGGCAGACAAAGTAGAGGGCCAGGTATAGTCAAGGCCAGTGAAAAGGAAAATGAAATG AAATAAAAGAAAAGAAAAGAAAAATCAAATCCTCCAACTTGTGTACAGGATACACCCGAAGCTTTGTGTATATAAAGG CCACTTAATATCTCCTCCAACCTAGCAACACATTCGAAAGATAAGTTGCGCTTAAATCCTCTCCAAAAGAGCTAATC
8	*E. grandis* Cellulose Synthase	CTGCTGAAATTCTCGAGGAAGTTGAGAGGTTCCAGATTAGATCTTTACCAAACAAAAAAAAACTATTGCTTATGCTAAA TTGGTCATTATAATAAGATTTTTAGAATACTCGTTGAGTATACTCAACTCAAGATATTATAAGTTTTCTCAATTGGTTTTT CTCCATTTCTTATGATCCGTCCACGAGCTTGGAGTCGCTTTTGAAGATGTAGCCAGCCCAACAGAACCGTTTCCTTCATCT TCCCGCGAAAGTTTCATGTCATCTCCCTCCTCTGCATCACGAACCAAACCTCTGCTCTCTCTCTCTCTCTCTCTGCTTCA ACACAATGACACCAACATCGCACCCTCCTCACCTTCCCAACCACCGCCATACCATCTCCTTTAAGCATTCCGATGAGTCC CTGATCCACCGCCTTCTCACTGAGCCTTCCCGCTCTCCCTCTTCTCGTCTCACTTTCTCATATAAAGAAGTGAAAGAATAC GAGGATACTCCACTTGGGTATCGCCAAGAACTCAT
9	*P. radiata* LIM Promoter	CCTTTGGGAATGAACTTTGAGACCACCTCCAACCCGGATTCTGAAATCCATCCAGCAATTCCAAAGTTCCAAACCGAAAT AAACATCCCACCATACCATGGCATTCGGAAAAAAGCTAGGCTAAGCTGAAAATCACTGTCATAACCCAGTAAGACCATG CCACTAATAGCAAGAGAACCATACACCAACATGCAAAGCCATGCATGTCCAAACCAGCTAGGAAATCACACATGCAAA

		GGGTTACCTGCAAGTATTCCTGTTGAAGTTGCTTGATCCTACTTTCTTTTCCTTGAGCCTTGCTTGCCTTCCTTTCCTTTGC TTGATTTTCCTTTCCTTGCTCCAAACTAGAGTGCTCTAAGAAAACTCTAAGTGACCAAGAGAGTGAGAGAGAGAGAGAA TAATGAGAGTCCAAACATGAACTTGACAAAAGCCATGAACTGATCCTCAGAAGTCATTTTATGCACGAGGCTTCTATTTT CTTCATTTTCCATCATTTTCCTTCAATTTCCTCATCACATGCAACGTGCGACTTTTCACCCCGTTTTCCTCCTAATTTCTTTT ATTTTCATAAATAAATGTGCCAAAAATGCCTcTTGCCTTAGCCTTTGCCAGTTTCCTTAGCCAAAACACACATCCAATGAT GCCCACTAGGATATCTTTGCCCAACATTAAGCCTGGAATAAATGTCTCTTAATCGTGGTCTTATTTTGCTTTTATTAACTT TTATTACATGAACTTTTCACTAAAGCTATTACAAAGATATATTTATTATGGCAATTATGTTTGATTTTTGAAGAGCTAGTA ACTTTTAGTTTATTATGGCCTTTTCCGTAAACTTATTTTCTTGAAAATCTCTATAAATCCAATGAAAAATTTATAGAATAT ATGTTGTGTTTTCTTCACTACCTCTAATAAATTTTTTACTTAGTAATCTACAAAGCCATTTATTAAAAAATTCAAGTTAAT TAAAAATTAATATCATTTCAAAAGTCTTTTTAATATAGTCAAAGTTTATTAAATTCTATGATGTATATTTCTTTTAAATAA ATGAAGAATCCATTTTTTTACTTAAAACCATATATTTTTTATAACGTTGATAAATAGCATGCATTTATATAAACAAATATA TATTTTTATAACGTTAAGAGATTGTTAAAACTTTTAAATAATTAATATTTTATTTATTGTTTTGAAAATGTCATGATTTCC ACCTACCTCGCCCATCAAATCTTGCTGCAAACCAGGCTTACCCAACCCCACACCCACAATATATTTTTGGGATCTGGTGC CCCCACCTTTGATCACAGTGAACACCATAAAGACAAATTATAAAGGCAAGGGGACTTGGCACCCATGAGGCAACCGAA AGCAACAAATCATTTTTTTCCAAAGAGATGAGTGTATGCCAACGAAGAAACACGATGAACCCACGTGTCATTGGCCAAC TCCCACTTTCGACAAAAAGAAGGAAATTAGAATTAAAAAGGCGAATAAAAATTGAAAGGCCATTTAAAATAGAAGGAA GAATAGCCTATATGGTAGATTTAAATGCTTTTTTGAAATCCGGTTACTCGCAAGATTATCAATCGGGACTGTAGCCGAAG CTT
10	*P. radiata* Pectate Lyase	AAACAGAGCAGATAACACTAAAAAGACCAACCCTGTTAGGAGGGGAGAAACAAAAAAGATCACACTAAAAAGACCAA CCCTCTTATCTAAACTTATTTTCTCTTATCTCTACCCCTTCTATTTTGAACCTTTATCATTTTGATAGAAAATATATGTTAA TAACCATTAAACCTACATTGTCAAGCTAGTGTAACTTATATGTTAATAACCATTAAACCTACATTGTCAAGTTAGTGTAA CTCCTTTGGTGGGGGTGGTTGTCTTCCTCTTCAATCTCATGCTATGACACACTTGTTTTTTAATAACATAGGCCGACAAGT TTGAGCCATTATCTATCTTGATTCCTCGAAATGATAAATAGATGTTGTCAGTGGACTTGAAAAAAACCAAGTAGGGAAC ACCACGTAATCTTTCCAATGGCATTAAAAGCTACTTTGAAATATGTAACACTTAGCAATCCTTCCAAGGCATTAAACCTA CTCTAACCTATGGAACACTTAGCATCCTTCCCACGGTTGATAATAAATGATTGATTCCTCAGAATAACAAATAAAAAAA AACTATAAAACTTACTCTAAAATATAAAATGAGTATGGAACACGTGGCAATCCTTCCCATGCTCGGCGGTAGCTACTCTC TCCAGAGATTTGAATAACACAGGCGCCGCAATTATGAGAGAGCAGTGGAGTTAAGACTTAGTAGCCATGGTTATTTTGA ACGCGTGGCAATTCTTCCAAAGGTTGGTAGTTACTCTATCCAGAGATTTGAATAACACAAATGCTGCAGTTATGAGAGA GTAGTAGAGTTAAGTCTTGTCAGCAATGATAGTTACGAACAACCGTAATTTCTGGCTATCTCTGTGTTTATTGGTCGTTTA CTTGCTACAGTGCTCTCACCCCACATGGTAACAGTGTTCGATGGCCATGATTTCTCCCCACCCCGCCAAACCTCTACGTTT TTATTCTTTTAATAACTCCTAATTTAATATATAAGAGGGGGCAAGGTGTTCATACAGATTCGTGCAAACGACCTGAGTTC AGCACAAGTTTAGTCATTCCATGCGAACTCGACTGGCTCACGAGATCCCTCGCTGCAGTTATAGATTGCAGGAATTAGCT TAGCAGCATTTCTATCTATGATCTTCTGCCACTTCTTCCCCTCTC
11	*P. radiata*	AAATTAGTCAAATCCAAAGCAGACAACTTGGGCTCTCACCTAAATTAACACATATACCCTACCAGCTTCCATAGTTTCCA

	Expansin	ACTTCCTTTCAATAAATCTATTCAAAAGCATGAAAAGCATGACTAAGGTTCAATTCCCAAGTTATGGACACCCACCTGCT CTAGGCATATAGGAAATCACAATCCAACTAACGACCAACTACCCAAAACTTTGAAGAAAATGAGTAAAGACTCCCCCAG TGATATTATAATTATATGGTCTCTCTAGAACCCTTTATTGCCCCTTCCAGTGTTATATTTAGTTCCCCATTTATATATCCCT TGACTTATGAAACCATTTAGGTGCATTAACATAGTCCTTGACTAACAAAAAAATTATTTAGGTGCAGTAGATACGGAAA GTAACCAATGATGCTAAGAAACTGTGCACGTACTTTAATGGAGGTATTACTTTTATTATGGTTGGTTTGGATACATTCAT AATGGAAGCATGTGCTCTTCATCGTTAAAGTTGTGGTGGGGCATTCCCCATTTTCCACGAGAAACCGAATCCCGGCGTGG AGACGACGACGAAATCGATGGATATTCGGTGGAAAATTCACAGTAAAATTCCTGGAGAAAAAGGTTGCCGAGGTAGTT GAAATCCAAACCGCCGAAATGAGCTGGAAACCCGCCTTCTGTCAGTTAGTTGAGTCATGACTGCAGCTGTCTCAGGTCTT ACACTGTAAAGGCACCTTAATGAGGCATTCATTCTGGCAGTCTGGCTACGGAACTTAATAGTACTTGTTATTCCTGCCCC AATATCTATTTAATAGGCATCCCCCCTCACTACTTCTTGCCCACAATCCCTCCATAGTCCTGAGCTTGAGACCATTTTTCT GC
12	*P. radiata* ACC oxidase	AAGGTTTGCTTGGACCAGCGACACAGGGAAAAACATGGCATGCGGGTTTGGATTAAGATGAGGCCCAATCTTAATTTGA TATGTTTGCCAAACCTTAGGTTGTTTATCTAATTTTTGATTGGATCTGATCTCTTGATGATTTAAGGGTTTTCCATGTTGAC ACGCAATTGTAGGTTCCTGGGCACTAAGGTCTACCATGTGGCGAATTTATCGAGAGTTGACAATTCTGGTACTGTTAGTG ATTTGTCACCACTCTACGGTCCCTGCAGATCTCAGATTTTTAATGGCTGCCTTTGATTATCTAAAGGCTAGCCCCTAATCG CGGCTATGAATGTATAAAGAATGTGTTCCAATGCATTAGAGTACTCAAAGACATGTTGAAGGAAAAGGACAAGTCAAG GGACATGAGTAATAACCAAAAAAGCACTTGGTCCTGACCATCTGTGTCTGATTCACACTGGGATTCACATGTTATTTAAG AAAAGTTGCATCAGTGCTGCAATCATCAAGCCATTCCTAATTTACCACCATGATTAGATTATTTTAATGCAAGAAAACGC CTATATAAGGAGAGCTGCAGGCCCCAAGGTAATGCAGTAATCAAACTTGAGGAGAGATTTGAGAGTGTTTGTGAAGGG
13	*P. radiata* 4CL3	AAATATAACATAATCTAACTATTGATGTACATTATTCGCCTATAACAAAATCTAAGTATTGATGTCACATTATTGGCATA TAACAAAATCTTTAGGATAACCCCTTAGTCAAGCTCTTGTACTTTCATGTTTATTAACCAATAAATCAAGCTGATATGGA ATAGCAGACGTACGTGGTAATAATAAATGGAGTGTAAGAGTTCGAACATTTTAATTCGGAGGGGCAGCTTATGTGGAAT ATCAGGCAATCATACAAGCTTGCTTTTGGGTAATAAAGACCCACATGTGGTAATAACAAGTGGATTTTAACAAACCAAC ATTTTGATAGGGAGGATAGGTGGCCTGGTAAGTTAGAATGTGCTAGTCATGCCTTTGAAAGAAGTTAGTTGTGGAAGTC AAACATGTTCCCCACACAACACACCTCACCACACAAAATGCTGGTAGGTCATGTGATTGATGGATGGGCATGTGTATCC TCCAAAAAAAATGAATATACACACTAAATATTCTATTGACATAATATACAAAGAAGATTAGGTCTATGGAAGAAGGGAA GGCGAAGGGGAAGATTGGGTCGTGGGGAAGATTGGGTCGTGTCCTGCTAGCACGTTGAATACCTACACGCCATTTCACA TCTACCCATCAACGTCAAATAGAGCATCCAAATCAGGGCGTGGTGGTGTGAGGGGAGAGTGAGGAGAAGAAGTTGAAA AATTCTGGCTGAAAATCCACCTAACACACGCTCACCAGCCCCTCAACGAGGGGCACCAATTATGAATAATAATAGCTAG AACAGAGCAGCAGAAGCAGAGTTTATATCTATCCATTGTCGTCTGTAAATTACTCTGTGAGTGTTTAGTGTTTTCTTCTCT TATTGATTTCAGGGGACAAGTAGGTGGG
14	*P. radiata* 4CL1 or 4CL2	AAACACCAATTTAATGGGATTTCAGATTTGTATCCCATGCTATTGACTAAGCCATTTTTCCTATTGTAATCTAACCAATTC CAATTTCCACCCTGGTGTGAACTGACTGACAAATGCGGCCCGAAAACAGCGAATGAAATGTCTGGGTGATCGGTCAAAC AAGCGGTGGGCGAGAGAACGCGGGTGTTGGCCTAGCCGGGATGGGGGTAGGTAGACGGCGTATTACCGGCGAGTTGTC

		CGAATGGAGTTTTCGGGGTAGGTAGTAACGTAGACGTCAATGGAAAAAGTCATAATCTCCGTCAAAAATCCAACCGCTC CTTCACATCGCAGAGTTGGTGGCCACGGGACCCTCCACCCACTCACTCAATCGATCGCCTGCCGTGGTTGCCCATTATTC AACCATACGCCACTTGACTCTTCACCAACAATTCCAGGCCGGCTTTCGAGACAATGTACTGCACAGGAAAATCCAATAT AAAAGGCCGGCCTCCGCTTCCTTCTCAGTAGCCCCCAGCTCATTCAATTCTTCCCACTGCAGGCTACATTTGTCAGACAC GTTTTCCGCCATTTTTCGCCTGTTTCTGCGGAGAATTTGATCAGGTT
15	*P. radiata* 4CL1 or 4CL2	ATCTTATGGAGTTTTTAAATATATATATATTTTTTGGGTTGAGTTTACTTAAAATTTGGAAAAGGTTGGTAAGAACTATAA ATTGATTGAGTTGTGAATGAGTGTTTTATGGATTTTTTAAGATGTTAAATTTATATATGTAGTTGTGAAGGAGTGTTTTAT GGATTTTTTAAGATGTTAAATGTGTATATGTAATTAAAATTTTATTTTGAATAACAAAAAATTATAATTGGATAAAAAAT GTTTTGTTAAATTTAGAGTAAAAATTTTAAAATCTAAAATAATTAAACACTATTATTTTTAAAAAATTTGTTGGTAAATTT TATCTTAAATTTAGTTAAAATTTAGAAAAAAAAATAATTTTAAATTATTAAACTTTTGAAGTCAAATATTCCAAATGTTTT CCAAAATATTAAATTCATTTGACATTCAAAATACAATTTAAATAACAAAACTTCATGAAATAGATTAACCAATTTGTATG AAAACCAAAAATCTCAAATAAAATTTAAATTACAAAATATTATTAACATTATGATTTCAAGAAAGAGAATAACCAGTTT CCAATAAAATAAAACCTCATGGCTGGTAATTAAGATCTCATTAATTAATTCTTATTTTTTAATTTTTTTACATAGAAAATA TCTTTATATTATATACGAGAAATATAGAATGTTCTAGTCCAAGGACTATTAATTTCCAAATAAGTTTCAAAATCATTACA TTAAAACTCATCATGTCATTTGTGGATTGGAAATTAGACAAAAGAGAATCCCAAATATTTCTCTCAATCTCCCAAAATAA ACCTAATTAATATAGTTCGAACTCCATATTTTTGGGAATTGAGAATTTTTCTACCCAATAATATATTTTTTTTATACATTTT AGAGATTTTCCAGACATATTTGCTCTGGGATTTATTGGAATGAAGGTTTGAGTAATGAAGGTTTGAGTTATAAACTTTCA GTAATCCAAGTATCTTCGGTTTTTGAAGATACTAAATCCATTATATAATAAAAACACATTTTAAACACCAATTTAATGGG ATTTCAGATTTGTATCCCATGCTATTGGCTAAGCCATTTTTCTTATTGTAATCTAACCAATTCCAATTTCCGCCCTGGTGT GAACTGACTGACAAATGCGGCCCGAAAACAGCGAATGAAATGTCTGGGTGATCGGTCAAACAAGCGGTGGGCGAGAGA ACGCGGGTGTTGGCCTAGCCGGGATGGGGGTAGGTAGACGGCGTATTACCGGCGAGTTGTCCGAATGGAGTTTTCGGGG TAGGTAGTAACGTAGACGTCAATGGAAAAAGTCATAATCTCCGTCAAAAATCCAACCGCTCCTTCACATCGCAGAGTTG GTGGCCACGGGACCCTCCACCCACTCACTCAATCGATCGCCTGCCGTGGTTGCCCATTATTCAACCATACGCCACTTGAC TCTTCACCAACAATTCCAGGCCGGCTTTCGAGACAATGTACTGCACAGGAAAATCCAATATAAAAGGCCGGCCTCCGCT TCCTTCTCAGTAGCCCCCAGCTCATTCAGTTCTTCCCACTGCAGGCTACATTTGTCAGACACGTTTTCCGCCATTTTTCGC CTGTTTCTGCGGAGAATTTGATCAGGTT
16	*P. radiata* Dirigent	AAACGCTTCATGCCCCAGAAGCCGCACTCGATGCTTTAGAATAAAATGGACCATTACCAGACTACGCGCCTCCAAAATA ACAAAAACGTGTATTAGTTAAACCCTACATAGCACTTAAAGCTTGTCTTACTATTATTTTACGTAATTCTGTCTTTTTGAC AGTGGATTGATTGGAACTTCCATTCTCGATACAGTTGTATGCGTTATGTGAACTGAACCAACCTCGGCCAAAATATGGGG AAGATTCACTTCAGAAAAGACAGGACAACCATCTCTGATTGTCGACATTAATATCGGAAAAAATTCAGTCAAATGATGT GGAAAGGTTCATCTACGGAAAATAAAATAGCTCTGAGATGACCCGTTACATTTAGTGCATAGCATCTTTGTCAACAAGA AGAAATTTCCAGTTGTAGGACTGGTCATCAATGGCCGTGCCTGCAACGCTTTTTCGCAACAGGAAACACGGACTAAAAA ACGCGGTCTATCTGTCATTTGACGGTACGTTTGGCACTGAGCCCGAAAAAATCCCATTGGTAGAATTTAGAAGAGGGAG CTTTCACTCGAAAATTCTGTACCACAAGCGGTGGCCTCACAATAACAAATTATTATACCCACATGGAAAATGTTAAATCG

		GACGGTCCGACGGTCGACCAAAGACAAAATTGATGAGAAAGTTTTGAGGGTGGGTGATAAAGTAAGCGCGTCTTTTCAC AGGCATCTGCATTATAAACCTGCAACTCCAACTTTCATCACAACAAATTTCATTTTCCCCTTCTCTGAGGC
17	*E. grandis* Laccase	AAAAAATTTATAACTAATATTGGTACAATTAGAAATTCTCTTGCCTTCCTTAATCTTGCTGTTAACTCCTCCATTTTAGGC GTAGACAACTATTTTTTTTCCACAAAAATGAAACAGTTCCTAAATAGACTAACGCCTTTTAAGCTGGTAGTAGACAGACC GACACAAAATCCTGAACAGGCATGTACCGACACAAAGAGATTCATTTCACGAGTAAATTTGAATTTCGACAACTAATTC TACACATCGGTAATCACGCAAATATATCAGATCGGCAAAAAGTTATATTTTAGACAGTGACGTGACATCTCAAGCACCC AATCCCTCTCAACAGGTGAAGAGCCATATTTTCATTACATAAAGGCATTTTTTTTTTTTAATTTTTAATAGGTGGTCCGAC CGACAAGATATTATTATTTTTCTATTTGCATGAAGAAGAAAAAGATTGGTTTTGACCACAATGGTTTGTCCTCTCGTTACC CATTTTATATTTGGCAAGTTTGGTGATTGATTGTAGAAGAAACACGAAACACACGAGCAAAAGTAAAGGACTCCAAACC CAAATTTTAATCCACAAACGAATTTACCCACATAAAAAAAGGGGAGATTATGATTAAATTCGTTGAATAATGCGACCCT TTAGGAGAAGGCTTATTAAGCAAGCATCGACGGAAGCTACACACTCCTTTTGGGGAGAGGCTAGTGGGTGCAACAACTA CGATTCGGGTAGAGCTAAGCTTTGTCCCCAGTGGCGGTACTGCCATGACCAGGGCTCTAAATCAAAACCTAATCTGCCA ACCTCAAAACAAACGCTGTCTCGCCCCCCCCGGCTGCGCTATATAATGCAGCCGATGGCGTCCTTCCTTTCTCGAACCCT AAGCAGATCAAGAGTTTGAGT
18	*E. grandis* Laccase	GTGAAGAGCCATATTTTCATTACATAAAGGCATTTTTTTTTTTAATTTTTAATAGGTGGTCCGACCGACAAGATATTATTA TTTTTCTATTTGCATGAAGAAGAAAAAGATTGGTTTTGACCACAATGGTTTGTCCTCTCGTTACCCATTTTATATTTGGCA AGTTTGGTGATTGATTGTAGAAGAAACACGAAACACACGAGCAAAAGTAAAGGACTCCAAACCCAAATTTTAATCCACA AATGAATTTACCCACATAAAAAAAGGGGAGATTATGATTAAATTCGTTGAATAATGCGACCCTTTAGGAGAAGGCTTAT TAAGCAAGCATCGACGGAAGCTACACACTCCTTTTGGGGAGAGGCTAGTGGGTGCAACAACTACGATTCGGGTAGAGCT AAGCTTTGTCCCCAGTGGCGGTACTGCCATGACCAGGGCTCTAAATCAAAACCTAATCTGCCAACCTCAAAACAAACGC TGTCTCGCCCCCCCCGGCTGCGCTATATAATGCAGCCGATGGCGTCCTTCCTTTCTCGAACCCTAAGCAGATCAAGAGTT TGAGT
19	*E. grandis* Unknown Protein	AAATTTTTTTTTTTTTTTGGGTGGGTAGTAGGATCTGTCAGAGTAAAGTGACTTAACGCCAATTCTCGACATTTCAGACTA ATAAAATATTTACAGATGCAACGTCTCACTCTCTCCTTGCAAAACCAGAAAGGGACAGCAAGCAAGAAGAGGGGGAAG AGAAGACTTGCGTTTTAAGCAAGGGGAGTGCTGACTTTTCAAGCGACTTAATTAATCTGTTTAGCACCCACTTTGGTTCG TTTGATCTTCTCGTGATTTATTATTTACCTATGTACAGCTGCGGTTGAAATGGCCTCTCTCGCTTAAATGGTAGTTTGTCCT TTTCTTGGGGTGGTTGCTTTGGAAATATTCTTTTAGAAGCAGGGGCAAAGAAATGGAGTGGCATCTGATGCTTCTTCAAC ACTTTGCAGCCATATCGAGAATATATACCTAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGGAGCAGTGGAGAAGAAG GAGAAGAAGAAAAGGGTCAGATCAGATCCAGTTGTTGGGAGCAAGT
20	*P. radiata* Cellulose-synthase like	CTGTATTCATCACTTTACACCCATGATTCCAAACCCTACACATTTACACTGATAACCAAGGGTTCAGGTTCTTTCCAATTC ATTTTAATCCAGGATGATAATAAATTTGAATAGCACAATAGCATATTCCAACTGaCATATCCCTACATTTGGGATCTCTTT CCACGTTATAAATGGCTTCAATTTAGGGATCCCTTTCCACATTATATAACTGGGTTCACAGTGGTTTGAAGATAGCTGTG GTTTGAAGATAGCTGTATATGTTATCAAAATGACAGCTCCCTTGCCAGGGACCATCGCTTGAATGATGAGATCCCGCCTG TAAGGCAACTTGCAGCATGATTATTTTACATCTGCTTGACCAATTATCTAACAATATACGCGGTGTCGTCGTTCGGTTAA

		ATAATAGTGAAACTTCCTCGTGTTGTCCCTGCAGTTACGTATGTCTTGTTCTTTTTTTTGTTTAATAaCATACAGCAGAGCA AGTGTTGGGTGAATAAATATTGGGAAGAAGCTGCAGCGTTCACGTTCATTCATTCACTCATCGTGAGCAGCAGTACATC AACAGTTCTTGAAGAACATTGATAGGTTGGCTATTTCAATCCTTTCATGGGGAATATTTAAGTCTGGATCCGAGC
21	*P. radiata* 4CL3	AAATATAACATAATCTAACTATTGATGTACATTATTCGCCTATAACAAAATCTTTAGGATAACCCCTTAGTCAAGCTCTT GTACTTTCATGTTTATTAACCAATAAATCAAGCTGATATGGAATAGCAGACGTACGTGGTAATAATAAATGGAGTGTAA GAGTTCGAACATTTTAATTCGGAGGGGCAGCTTATGTGGAATATCAGGCAATCATACAAGCTTGCTTTTGGGTAATAAA GACCCACATGTGGTAATAACAAGTGGATTTTAACAAACCAACATTTTGATAGGGAGGATAGGTGGCCTGGTAAGTTAGA ATGTGCTAGTCATGCCTTTGAAAGAAGTTAGTTGTGGAAGTCAAACATGTTCCCCACACAACACACCTCACCACACAAA ATGCTGGTAGGTCATGTGATTGATGGATGGGCATGTGTATCCTCCAAAAAAAATGAATATACACACTAAATATTCTATTG ACATAATATACAAGGAAGATTAGGTCTATGGAAGAAGGGAAGGCGAAGGGGAAGATTGGGTCGTGGGGAAGATTGGGT CGTGTCCTGCTAGCACGTTGAATACCTACACGCCATTTCACGTCTACCCATCAACGTCAAATAGAGCATCCAAATCAGGG CGTGGTGGTGTGAGGGGAGAGTGAGGAGAAGAAGTTGAAAAATTCTGGCTGAAAATCCACCTAACACACGCTCACCAG CCCCTCAACGAGGGGCACCAATTATGAATAATAATAGCTAGAACAGAGCAGCAGAAGCAAAGTTTATATCTATCCATTG TCGTCTGTAAATTACTCTGTGAGTGTTTAGTGTTTTCTTCTCTTATTGATTTCAGGGGACAAGTAGGTGGG
22	*E. grandis* *Euc ATHB8*	AAACGGACAGGAACCAAACTGGATCGGATCCAATTCCTAGTCCTAAAACCAACCAATCCCCACTTTCTAATTTTTGGAAT CGGTCCTATAGGTTCCATTTTGAAATCGATCGCCCTTATATGAATGAAAGAGCGCTCACATGTACCGTTAGATGGTATAG ACCTAATAATCTGATAATCTGATGGCTCATTGCGTTTTGAGCTCACATGGAGCGAGATTATGTAATAATGACGTCAGGGA GAGGAGAGGAGAGAAGATGAAGAGAAAGCTGTGGAGAAACAAAACACAAGGCTCGTTGGAAGCAACGTAAACAACAG CAAACAACATCAACAACGGCGACAAAAGAAGAGAGAGAGAGAGAGAGAGAGAGGAAACAAAAACAAAAGCAAAA GTTGGGGAGTGAAGAGGGGAAAAGAAAGATGATGTGAAAACAAACCAAACTCTCCTTTTCTTCCACCTCTCATTTTCTG TCTGGTATATGGGGGTCTCTCTCTCTCTCCCTCTCTCTCTCTCTCTACCTTCTCTCTCTACTTTCTCTTTCTTAGGGGGGG GCGTCCCCAGGGTCTCCGATCCCAATATCATTCCCCCCCACTCTTTTGCTGCCATATACATACAAAAAACCGAAGCTTGT GAACAACCCATCTCTCTCTCTCTCTCTCCCTCTCTCTTTCTGCCTGCGAAACTGTGTC
23	*E. grandis* *Euc Ted 2*	AAAACAATGTAGCTTCTCTGTGTTATGAAAACTAACAAAAGGGCACATCTATTTCTCCATGACCATTATATTCGGAGGAG CATGGTCAAACTTAAATCAAAATTTATTATCCATAACTTACAAATTTCCAATTTAGCTAAACTCAAATCCCAAAGTATAG CAATTCTGTTAAAATTTTATCATCCGATAGTATAGAGCAATATTTTATAATACTTACATTGCTCAGCTCAATTACAAATTC TATTTGTCCACAAATTCAAACATTTTAATGATGCATTCCACATAAAACCAATGGTTTGAGACACCTTTTCAAAAAAAAGA AAAAAATACACTAGCATTGCTTAGACAAGTTAATCAATGAAAAATAACTTTATCTTGTTTTTAATTAAGGATGAAAAGG AGTTACAAACGCTTGTTTCAAGATAAATATTTTTCAAATCTTTAATATTACAAGAAATAAACGGACCTTCTTATCAACCA AAAAAATGTAACATAAAAGGAACTTACCAATTTGATTGGACTCATTTATTGATTTTTGGAAAAATGTCGCAAATTTTCGT TGAGTTTTAGCTCCATGTACAATTTAGTCATTGAACTTTTAATTTATTCAATATAATTCATGAACTTTCTATACATATTTA GTCCATATAAAAATTAAGGGACCAAATTGAGTATTCACCAAAATTTTAGGGAAAATATTGAATAAATAAAAAGTTCTTG GACCAAATTTCATATTGAAATAAAATTCATGGACAAATCATTATTCCTTGATTAAACTTTTTTATGTAGACACCCGTAAA TACAACCTGCCAAGGTTTGTTTGCAAGGCGTTTGCAAGGCGTTTGCACTTAAGCGGGACGGAGGCGTCACCAGTCAATG

		GGCATGTCCAGTGGCTTCCCCGGCTTGCGAATAGGATGCTTCCTGAATCATCTCC
24	*E. grandis* *Euc cyclin*	AAAGTTTCTCTGTAGAGAGAGGGAGGGAGATATATCTGCGGTTTGCGTCTCTATTTCGCTTGTGCAGTTTTACTACTCCC CAAACACACACACACTCTCTCTGTTTCTCTCCTTTTTCCCCAAATCAGAAGAAGAAACGACAGTGTAGTAGTGCAGTTTC ACTACACCGTCTATACTAAGGGTAATCGTTTTTTTGAAAGCACATGCATATAGCCGTTGGAAAGGGGAGGGCACCGAGA TCGAATCGGATGGCTGATCCTCACTAGCCGTTAGAGAGAGAGAGAGAGAGGGAGGGATAATCATGTGCGGACATATAT CCGCAATTTGCGTCTCTATTTCGCTTGTGCAGTTTCACTACTCCCCACACACACTCTCTCTCTCTCTCTCTCCTTTTCCCCC AAATCAGAA
25	*E. grandis* *Euc cyclin*	CCTTGTATTTCCCCAACATTAAATGAAAGCCTACATCCAAAAACGTGGACCCGGCATTAAAGAAAAACCCCATCATCTC ATCCCATCCTTTATTTCAACCCTAAAGTGAAATTAAGATATAAGACGAAACCACCCCCAACCCCCCAAAAAAAAAATAT TAAGGGAATTCGTTTTTTTGAAAGCACATGCGGAGGTAGCTGTTGGAAAGGGGCCTCTACGTTCGGAAGGAATGCGACC ATTCCATCGAGATCAAATCGAACTACTGATGCTCACTAGCTGTTGCGTTTAAACCTTCTTTGTAAAGCGATAAGGGAATT CGTTATTTTGAAAGCACATGCGGAGGTAGCCATTGGAAAGGGGCCTCTACGTTCGGAAGGAACACGACCGTTCCACCGA GATCGAATCGGACCGTTGATGCTCACTAGCCATTGTGTTTAAAGTTTCTCTGTAGAGAGAGGGAGGGAGATATATCTGC GGTTTGCGTCTCTATTTCGCTTGTGCAGTTTTACTACTCCCCAAACACACACACACTCTCTCTGTTTCTCTCCTTTTTCCCC CAAATCAGAAGAAGAAACGACAGTGTAGTAGTGCAGTTTCACCACACCGTCTATACTAAGGGTAATCGTTTTTTTGAAA GCACATGCATATAGCCGTTGGAAAGGGGAGGGCACCGAGATCGAATCGGACGGCTGATCCTCACTAGCCGTTAGAGAG AGAGAGAGAGAGGGAGGGATAATCATGTGCGGACATATATCCGCAATTTGCGTCTCTATTTCGCTTGTGCAGTTTCACTA CTCCCCACACACACTCTCTCTCTCTCTCTCTCTCCTTTTCCCCCAAATCAGAA
26	*E. grandis* *Euc F5H*	AAAGATAAAAATAGTGTGGAAAATAGATTTGAGAAGTGTTCATATATTTCGATTTATCATAGCAAAGATTTTATCGACCT ATTTTAGGCTTTATAGTGTGACTATTTAAGATAACGAATATTAATCGAGAGATGCACAATTAATAAGAGATATTCTCACG ATCTTGAGATATATAGAAACCGACAGAAAATATATTGATTATCTCTAATATAGAATAATATTCTAGAGAAGTATTGTAAT TGTGACCACCAACTAAAATGGGGCAGACAAAGTAGAGGGCCAGGTATAGTCAAGGCCAGTGAAAAGGAAAATGAAATG AAATAAAAGAAAAGAAAAGAAAAATCAAATCCTCCAACTTGTGTACAGGATACACCCGAAGCTTTGTGTATATAAAGG CCACTTAATATCTCCTCCAACCTAGCAACACATTCGAAAGATAAGTTGCGCTTAAATCCTCTCCAAAAGAGCTAATC
27	*E. grandis* *Euc cellulose* *synthase*	CTGCTGAAATTCTCGAGGAAGTTGAGAGGTTCCAGATTAGATCTTTACCAAACAAAAAAAAACTATTGCTTATGCTAAA TTGGTCATTATAATAAGATTTTTAGAATACTCGTTGAGTATACTCAACTCAAGATATTATAAGTTTTCTCAATTGGTTTTT CTCCATTTCTTATGATCCGTCCACGAGCTTGGAGTCGCTTTTGAAGATGTAGCCAGCCCAACAGAACCGTTTCCTTCATCT TCCCGCGAAAGTTTCATGTCATCTCCCTCCTCTGCATCACGAACCAAACCTCTGCTCTCTCTCTCTCTCTCTCTCTGCTTCA ACACAATGACACCAACATCGCACCCTCCTCACCTTCCCAACCACCGCCATACCATCTCCTTTAAGCATTCCGATGAGTCC CTGATCCACCGCCTTCTCACTGAGCCTTCCCGCTCTCCCTCTTCTCGTCTCACTTTCTCATATAAAGAAGTGAAAGAATAC GAGGATACTCCACTTGGGTATCGCCAAGAACTCAT
28	*E. grandis* *Euc CAP*	GTGCGGACACGTGTCCCCTTATCCCGCCCAAGACCGCGCAAAACCTGAAAATCCTCACTATTCCCTCACTTTCGGCGAAT TCGAAACAGCGCATAAAGGAACACGGAAAGAACATTCTCTACCCCAAGACGACGACGACGACGACGACGACGACGCCG CGCCTTATATAAACCATCGCCACTCCTGGCCATTCCCTTCTTTCTCCCCAGATCCAAT

29	*E. grandis* *Euc microtubule associated*	AAATTATGCAATTTCTTAATCAGGCCTAGCTAGAAACAAGGGCAAGGAAAGCCCCCGACGGGCTCTTATCTGCTGACGT GGCACGCCGTGGGTGGGCCCCCCGGGTCTTCCTTCGACGAAACCTCATCGTAGACAATCAAATCCTCCTCTCGATCATT ATTGCAAAGCCAACACCCAGCATTGAATCGATCCCCACCTTCTCCTCCTCCTCTTGATCCTTTTGTCCCGATGATGA TGGGTATCTGATCAGCCGATTCAATCCCATCGTCTCCTTCCTTCTC
30	*E. grandis* *Euc glycosyl transferase*	AAAACCACAAATGGCCGCGGGACGTCACAATTTTTTTTCCTTCTAGAAGCTCTATAGTCAAAGCTGATCTATAAATTTT TGGGAACCACAACCACCATGTCTCGCCACCTTCGCTCGAACCTTATCACCACCACCGCCCTTGAGCCCTCCTCCATCAAC TCTTCTTC
31	*E. grandis* *SAD*	CTGTCACCTCTGGCTGGTCGCCGAACCTCAGCGACCGACTGGAGGAAGAAGAGGAAAAGAAAAAAATAAAAATAAAAT TCTAAAATATTAAAATATTATTAAAAGTTGTCCACGTCAGCGTTGAGGCCACGTTAACTAGCCGGTGTCGAGTCAGCAA AATTCGGCCAAAATTGGCACAAAAAAAGGTTTAGGACTTTTTTGACGCTTTTCCCGTCATGAGCCTAAATAAGAAATTTT AATTTCTTCATACCATACCAATTATTTGATATGAGATTTTTCTAACTAATTCACACATCTATGCTAACGCTACTCGCTCAA AAAGCGCTCAAGCTGAAGCCAAGTTTCAAGCATCAAGCTTATAAGCCGAGCCAAGCTCGAGCACGGTGCTTCTTTTCTC GGCCTGACCCGATTAGACTCTTGACTGAACATGACATATGAAATTGCAGAGCATTCAATTTAAAAGATTGTGAAATTTCT GGGCATTTATTTACCTCCCTGTTAATGATATTGCAGAGCATTCAATTTAAAGATTGTGAAATTTCTGGGCATTTATTTACC TCCCTGTTAATGATATTTTTATGGAATAGCGTGCAAAGAATTCGGGTGCATAGTGTTGTCCTTCTCCCAACGCCCCCTTAT ATAATCTCCGAACGGAGCAAGCATTTGCTCTTCCGTACCCACGGCATTTTCCTTCTCGTGACCTTTTCCCGAGAAAACAA GAAGAAGAGAAAATCCTTCCATTGCATCG
32	*E. grandis* *B-expansin*	CTGACGCCTAACATGTACCTTAACTGTAATGTAGCAGCGCAGGTCGTCATAGCAGAGGCCGGTCATGCTGGTCAGGTAG TTACTTTAAAAATCTGGAAAGCTTCTTTGTTGTTGTCTTTGCTGCTTTTCTTCGCTCTTTCGGCGAACTTCCCAGTCGATTC ATCGGTCTAAAGAATAGACACGGAGGTTATCGCAAACTTATGCAGAGATTCCTTGCGGTACGCAAATGCATGTTTAATA GATCATTATGTTAAATAGATAATATAGTGACTTTCAGGATCCGTTTGTTTTGCAAATTTTTTTTCCTAAAATTGGTAACAT GCAACGCTTGAAATTATCAATTAGCGAAAAATATTATTATCATAGAGAACAATTTATATAAACCTTCTCCCAGCCTAATA AGCATGCCTGGTTCTCTAATATCAAAGAAAAAGAGGAGCTAGATCTCGCCTTTAGAATGATTTGAAGTAATTGCAGTTA GCTTGAAGACATTCGTAGATGTCGATTGATCAATGCTTTTGGAAGTACTAGAGATGCGCACGCATACGTGCGATATCCA AACTATTTCCGTTGACCCTCACGAAAATCTCCGTACAGACCGTTGTTGCTAATTCTTTATTTGCCGTAAAATCTGCATGAA TCCATAAATTCAATGATTCGAACGTGACGCAGAGGAAGTTATGCATTCCAAAAGATAGCATTTATTTTAAATAAAGAAG TGAAGATTACAATATCTTAGGTGCCTATTTATAGAGAGGTCGTCATCTAGAAAATAACCAAGTAACCGAAATTGAATAA CAAAATTAAAAAATATATATTGATAAAAAGGGAAAGTTATCAAAATACAACTAGAAAATCTCCAAAATGTGTTTGAAAT CTGTGATATCTCGGATTTGTGGGATCGCTTGCTCTCATGACGCTCTAATGTTTCCATAAAGGCATTTGCGGAGATTATTGT GTCGGATTATTCAGCTTGCAAGAAAAGTTATAGTGCGAGCAAACCATATAAAATAACAATAAATAATGGCAAAAACTAT CGCCGAAAATTCTCCAATGACGACAAGGACTCCGATTTAGTGGAATTTTGTGCTGTCAATTTGACTATAAATACCCGCCC GTTGTGCTCCCAAATCGAGTGCAAGAAATGAAACTCCTGACCAA
33	*E. grandis* *Putative Laccase*	GTGAAGAGCCATATTTTCATTACATAAAGGCATTTTTTTTTTTAATTTTTAATAGGTGGTCCGACCGACAAGATATTATTA TTTTTCTATTTGCATGAAGAAGAAAAAGATTGGTTTTGACCACAATGGTTTGTCCTCTCGTTACCCATTTTATATTTGGCA

		AGTTTGGTGATTGATTGTAGAAGAAACACGAAACACACGAGCAAAAGTAAAGGACTCCAAACCCAAATTTTAATCCACA AATGAATTTACCCACATAAAAAAAGGGGAGATTATGATTAAATTCGTTGAATAATGCGACCCTTTAGGAGAAGGCTTAT TAAGCAAGCATCGACGGAAGCTACACACTCCTTTTGGGGAGAGGCTAGTGGGTGCAACAACTACGATTCGGGTAGAGCT AAGCTTTGTCCCCAGTGGCGGTACTGCCATGACCAGGGCTCTAAATCAAAACCTAATCTGCCAACCTCAAAACAAACGC TGTCTCGCCCCCCCCGGCTGCGCTATATAATGCAGCCGATGGCGTCCTTCCTTTCTCGAACCCTAAGCAGATCAAGAGTT TGAGT
34	*E. grandis* *Euc unkown protein*	AAATTTTTTTTTTTTTTTGGGTGGGTAGTAGGATCTGTCAGAGTAAAGTGACTTAACGCCAATTCTCGACATTTCAGACTA ATAAAATATTTACAGATGCAACGTCTCACTCTCTCCTTGCAAAACCAGAAAGGGACAGCAAGCAAGAAGAGGGGGAAG AGAAGACTTGCGTTTTAAGCAAGGGGAGTGCTGACTTTTCAAGCGACTTAATTAATCTGTTTAGCACCCACTTTGGTTCG TTTGATCTTCTCGTGATTTATTATTTACCTATGTACAGCTGCGGTTGAAATGGCCTCTCTCGCTTAAATGGTAGTTTGTCCT TTTCTTGGGGTGGTTGCTTTGGAAATATTCTTTTAGAAGCAGGGGCAAAGAAATGGAGTGGCATCTGATGCTTCTTCAAC ACTTTGCAGCCATATCGAGAATATATACCTAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGGAGCAGTGGAGAAGAAG GAGAAGAAGAAAAGGGTCAGATCAGATCCAGTTGTTGGGAGCAAGT
35	*E. grandis* *Euc unkown protein*	ATCATTAATATCATTAGAAAGATATATTACATTTTAAAGATGAATAAACATTTGAAATGTTTTCTCTACAACTAAAAAAA AAATCATTGCCTCTACAACTAAAAAAAAAGATCATTGCCCATTATGACATTTCATTTTTTTTCTAATCACATCAAATTACTT TAGAATAACTATCCAGCTGCCAAAAAAAAAATAGTATTGTATATCTAAAATAAATATATTGACAAATGCCAACTAAATTA GGTATCAACGAATACCTCTTACTTTCCTACAATCGAAGATGTAAAGACTAATGTACATTTCTTCATGATTATGTACTAAT CGATTACAAAAACCAACATTTTTTTTTAGTTCTTGAATTTCTTTTATTTAGGAACAGTATCCACCAAATATGTTCTTTAGC TAAAGAATGATATATATTATTTTTAAAATTGCGATTGGATTCTTCATATGTTATATCTTGTTCAAATATTATTATTTTGATT TGATTTTCAAAATAAAACAGAAAAATAAATCTCATCTCGTTCCTTTTTTCAATAGTGAAAATCCCACCAAATTCATTGAC AAAAAATCATGAAAACAGTAAAGCTTGTATTTTCATTCCCAACTTTAAAACTGGTGGGTGACATTCCAAATGATCATATG GTCATATACTAATTTTCCCAATTTCTGAGCGTGCTCAACGTGATTGTACCCTTTTATTTTCGGATCATGCCAGGTCAATAG AGAAAATTTATCAAAGATTAGTTTAATGATGAATTGGGACCAACCTCTCAAGTCCATGAAACTGCCGTATGCGCAACCG TAAGCTCCGTTCCAATTTTTCTAATGATTCAAGGAAAAATAAATAAATAAAAAAGTCCATCGTCGATGTGACATTTCGCC TGCGCTCTCCAGCTACTTAATCAATCAATATATACGAATTATTTAGCACATGACAGCATTTTTCCCTTTTTCCTGGCGTCC TCCTAGGGTGGATCCGGACCGTGGATCGAACTACAGGAGTGGCGGGGCTCTCCGCCACCGACAGCAAAGTCAATATCAA TCATCGATGGCAGTCGCTTTCCGGACGATTCATACTCATCCGAGTCCATTTCCCACTTCAACCTCAAGTCCCTCCTCGTCC ACAAATGTAAAAATGAAAAAATGGAGGGCAGATTAGACTGAATTTTAGCTGTACAACACATGTTGCCTGTGCTTCACGT TCAAGATCCACGGTTGCTTTGCTGTTGCACTCGCACCAACTGTACTGAATCTCCCTTTATTTCTCTCTTTTAATTTTTTTTT TTTTGGGTGGGTAGTAGGATCTGTCAGAGTAAAGTGACTTAACGCCAATTCTCGACATTTCAGACTAATAAAATATTTAC AGATGCAACGTCTCACTCTCTCCTTGCAAAACCAGAAAGGGACAGCAAGCAAGAAGAGGGGGAAGAGAAGACTTGCGT TTTAAGCAAGGGGAGTGCTGACTTTTCAAGCGACTTAATTAATCTGTTTAGCACCCACTTTGCTTCGTTTGATCTTCTCGT GATTTATTATTTACCTATGTACAGCTGCGGTTGAAATGGCCTCTCTCGCTTAAATGGTAGTTTGTCCTTTTCTTGGGGTGG TTGCTTTGGAAATATTCTTTTAGAAGCAGGGGCAAAGAAATGGAGTGGCGTCTGATGCTTCTTCAACACTTTGCAGCCAT

		ATCGAGAATATATACCTAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGGAGCAGTGGAGAAGAAGGAGAAGAAGAAA AGGGTCAGATCAGATCCAGTTGTTGGGAGCAAGT
36	*E. grandis* *Euc Csl-9314*	AAACTCATACTCTTGATAAGATGCAGACATTGCTGGCGTTCAACAAGGAAAAGAAAAAGAAGGAATAAGACAAAGTGA AAGAGAAAAAGGAAAAAAAAAGTAAAGTAAAATAAAATATCATTAAAAATTGTGTATGTTAGGGTTGTTAGGCATTTA TGTCCGCACCAATTGACGCATTTATGTCCGCACCAATCGAACCATTTATGTTCGCACCAATCGACGCATTTATGTCTGCA CCACTCATCTGCACCAATTGGCCAAAATTGGCCAGAATGATTGAATTGACATAATTGCAAAATATCTAAGACTGAACAA GCAAAAAAAAAAGTTATGACCGAATTAGAAAAATTACAATAGATTTATGACTTTTTTTGTAATTCCCCCCACCTAACTCT GTCAAACCTGCTAATATAGACTAATTCATTCATATATTTATATATACACACTCATAGGTTGATATATGAATATGGGGGTA CGTATAACCCTATGTGCTAAAATCTTGGAGAACTTCCTATTCATATCAGAAGAAGAACCGATCCTGT
37	*E.grandis* *Euc CAD*	AAAACAGATTGTTTTAGATTGATAACGTTTTCCTATCATGCCGGCATCATCTCAATTTTGAATTATATCGGAGCATTAAAT ATAAAAGTTAGGTTACGGATGAATGATAACGCAGACCTAGTGAGAAAATTAGTATAATCACGATAAAAATATCCATATA GACATCACAAAAATGCCGCCCGATCTGATGAAATCCGACAAATAACACAAACATATATATGTCCAAGACTTGGACTTCA AGTCGACATGCTTGTGCATGCACAATTTTGGGCCATAAAATTGGGCATGTGAGAACCTCAAACCGTTAAGAGATCAGGT ATTTACTTTGTTTGTCGACTGACGAGACGTGCACGCATTTCACACCCTCTTCTCATTGATCTTCAAAGCTTTTCCGAACTC ACGATGGTTCCAGAAAGGCGATGTTTTGCTGACAGAGGGAGCGTTCGATGGAGCTTCTCCATCACTTAATTTGTCCCTTC AAGATGAAAAAAGTAAGAGGTCCACCGTACCAAAACATTCTTCCACCCAGAAGAAAACCACAGTAGCTGGAGGGAGTC AAGCATGTCAGAAGCACAGAAACTGGGAATGGCTAAAAAGCAAGTCTTGACCCTTAACCCACCCCACTGGTTCACCTAC CGCACCTCGGGTTAGGTATTGCTTGCTGAGGTGTCACTTTTCGCCAAAGTCATGTCTCTCTTTTGGAATCTTCTTATTGGT CCGTCTCGTTTCCTCGTTGCTGGATGCTGGTAGCGTTTTTGTCCATATATATATGCAGTCCATATGTATCCCCGTCACTCC TCATCTATGCTCCTACCCGGCAACTTCCCACTACGATAAGCAGCAAGTTTTCGGCTCTGT
38	*E.grandis* *Euc CAD*	ATCAGGTATTTACTTTGTTTGTCGACTGACGAGACGTGCACGCATTTCACACCCTCTTCTCATTGATCTTCAAAGCTTTTC CGAACTCACGATGGTTCCAGAAAGGCGATGTTTTGCTGACAGAGGGAGCGTTCGATGGAGCTTCTCCATCACTTAATTTG TCCCTTCAAGATGAAAAAAGTAAGAGGTCCACCGTACCAAAACATTCTTCCACCCAGAAGAAAACCACAGTAGCTGGAG GGAGTCAAGCATGTCAGAAGCACAGAAACTGGGAATGGCTAAAAAGCAAGTCTTGACCCTTAACCCACCCCACTGGTTC ACCTACCGCACCTCGGGTTAGGTATTGCTTGCTGAGGTGTCACTTTTCGCCAAAGTCATGTCTCTCTTTTGGAATCTTCTT ATTGGTCCGTCTCGTTTCCTCGTTGCTGGATGCTGGTAGCGTTTTTGTCCATATATATATGCAGTCCATATGTATCCCCGT CACTCCTCATCTATGCTCCTACCCGGCAACTTCCCACTACGATAAGCAGCAAGTTTTCGGCTCTGT
39	*E. grandis* *Euc LIM*	AAACACTTTCTGTAAACTTATTTTTGCAAACAATCCAAAGCCAAAAAAGTAAAGAAACTATTTTCAGATAGGAAATTTTT CTCAAAACAAGGATCGTCGATGGGACTGGAGCTCTCAGCCCAAAAAAGAAAAAAAGAAAGGTAATGTGATGTAAGAGA GAGGAAAGTAAAGTTGAAGAACGTGTATGCAAAGCGACATGATGGGGGAGAGCATTTGATGGACAATCATTGGGCCAA CTCACATGAAGTCCTTACAACAAACAGTTGGAGGACGATGCAGCTCCAGCTCGATTCAGCGACTCCAATTATATTTCCCT CTCTGGTCCTCTCCTCCTTTCCATGCGCAATCCAGCTAAGTTTCTATTCCATGGCCCCTTTGCTACTAGGGTCACATCTGC CAGATATTTTTCTGGTATGCAGCTAAAAGCATAGTAGTGCCCTTTGGAAAAGTTGATCATAGTAACTGGGCTGGTCCAGT TTAATTAGAGCAATCTATGATGAAATTACTAATGAATTTTTGGGAAGTTCGGTTTTTGGTTTCTCGGAATTTCTCACCAAT

		ATCATTGCTTCAATATTAGTTAAAATAGACGACTGAAAAGATCATGATAGATAAAAAAAAGGGAGTGGCCAAATTATTT TTCTCTAATTCTTACTTAACTTAAGCTTCATGCATGCTGCCCATCTTGTGTTTGGTCATTAACTAACCTAGAAGGAGGGGG GGAAAAGGTAAAACATGTCATAAAAGGTTTAGTTAGACCCTTCACCCAAAATGATTGCCCAATGCCACCACTTTAATCA TCAACTTTCCAACCAACACTTGTTTTTTGGCTTCCCTTTCTTATCCTCCATTCTCCTCTCTCCT
40	*E. grandis* *Euc 4CL*	ATCAATGAGTGAAAGGGGGCGGCACAAGAGAGATATACTTACACATGCTCCCCCTAGACTAGACGACAGACGCAATTT ACACATGTCCGAGACATACGGTCATGAAATGGGAATTCTGATGTAGAAATAGCATGAACCCATTTAGCAAAGAATTGAG AACTGGGCCGGAACTCTGCTCGTGTTAACTAATCCAAGCGTCGGTCAAGCTGTGCGCACGCATGGGTGGAAGGGGGCG GGGGTAGGTGCACAGGGAATTTGGTTTGGGGGTTAGAGTTGGTCAAAAGCCGAAACGGTGTTAGGCATTGGGCTTTTTG GCTTTCGGCTTCAAGACAATTTGAAGGGGAGATGGGGCGTGCCATCTGCTCTCCCCCTGCCATATGACCCATCATCCCCT CTCCATCTCCATCTACCTCTACCTACCCCCGCCCCCTTCCTCTTCTTCTCCTTTTCTCTTTCCTTCTTCGAAAAATTTTAATT TATTAAAATATTTATTGCCCCTCCCCCTCCCCCTCTCCAAAACCGAATTTAACCCAACCCTCTCTCTTTCCCTCCACCCAA ATCTCTACACATCATCATCATCATCATCATCATCTCCTCCCCTCCCTTTTCT
41	*E. grandis* *Euc CAD*	AAAATCACTTAACGGCTTCACCCAATATACTAGTTATCTCATAAGTGGCAATCTAAAAAAAAAAACACTATAGTTACGT CGATGAAAGGTCCGACTTATCTGTTCGAAATCAGAACCTGAATTTCTATTATTGATCTAAACAAATCACGTCGAGTGTGA TCTAGTTTATGAAAAATACTACAAAGAAATGAAAAAAAAAATGTTAAATTGAATGCAATTTATTAGCAATGGGTTTGAA AATTAGTAATAGTATATCTATTGTCATGCAAGATATGAATATTTTAGATCCTTCTAGAAGCACGGATAACTTATGACTCG ATGTTTTCTTAAATCTTTGGACACTTGTCATTTTTTCATAGAGAAGCGACGAGAAGATCTTTCGCGGCTGTTTCACCTACC CCAACCTTTGTCCCTATGCATCTTGGCTGAGATGTCAACCTTAGGCTTCCGACACCTTTGACTCTCTCTCCTCCATCGTCC TCATCTCTCTCCTGTATA
42	*P. radiata* *Cinnamoyl-CoA reductase*	AAAAAAGTTTCCCAATCTCTAAGCAACCATAAAGCTCAACCACTCTCTGTCCTGTGCCCCAACGTCTACCAGACGATTAG GTATGCACTGCAGTTCTTCGTCTGTCATGCTACCAGACAGTTAGGTAACCACTAATGTCTTAGGTGGTGATTGATATTGA TGTTTCTTCTGCAAACATGTGAATCAATGTGTATCGCTGGAATATGACACTGTGGATCACTGGATATACATAGAGAGATC TGCTCTGTCCATTTTTAACAGATTCATCTCAATTTTCTTGTTCCAATGTCAACATTTTCTCAACTGCTCTGCCCCATCTTTA TTAAAAGGGAACATCTACCCTGCATTTCCACACTCCAATC
43	*P. radiata* *Cinnamoyl-CoA reductase*	CCTATAAAAAAAGATTTTATTAAGAGCATTTGGAAAACTATCATCTTTCCAGGACCATAAAACTATTTAATAGTTCAATA AAGATGAAGTAGTTACTATTTAATAGTTTAATAAAAATTAAGTAGTCTAACAGTTATATAGTTATATATATGTGTGTGTT TTGGGTATGTTTTCAGGTTGAATGATGTATAATTGAGTAAGGATTTTTTTTGGAATTAGTGAATTTTTTTTTTTCAGAATA ACAATTCTATATATATCATAAAAATAAATTTTAAATAAAAAAAATCTAAATAAAAATTATTTAAAAAAACACTAAAACC ATTAGTATACCAACACTTCAATTTAATGATGGATAAAATAATAAGCTAGCTCTGCTTAACATTACACTGTGGTGAGTTTG ACATGAAAAAATAGATCTCTGCTTTCAGAAGTACGCATTTTTAAATTTAAAAAAGTTTCCCAATCTCTAAGCAACCATAA AGCTCAACCACTCTCTGTCCTGTGCCCCAACGTCTACCAGACGATTAGGTATGCACTGCAGTTCTTCGTCTGTCATGCTAC CAGACAGTTAGGTAACCACTAATGTCTTAGGTGGTGATTGATATTGATGTTTCTTCTGCAAACATGTGAATCAATGTGTA TCGCTGGAATATGACACTGTGGATCACTGGATATACATAGAGAGATCTGCTCTGTCCATTTTTAACAGATTCATCTCAAT TTTCTTGTTCCAATGTCAACATTTTCTCAACTGCTCTGCCCCATCTTTATTAAAAGGGAACATCTACCCTGCATTTCCACA

		CTCCAATC
44	*P. radiata Phenylalanine ammonia-lyase*	AAAACTAATTTTCAAAATATGAGGAAAAAAGCGAGACCACGAAAAAATCATTGAAAAAGACCTTGCAAAATTCAGGAC TTGCTCTCACCAACCTCGCCAGGACTTTGACCGTGCTCATGCTTGTGTCATGCTTGCATATCTATACGTGTCACATCGACC GTCCGATCTATCATGAAAAGAACGGTCATGATGAAATCTCAACTAAACCCACTGCGTTAAATTTTCGAACAGTGAGAAA GTAATCGTATAAATACCCCTAAGCTCTTAGACCGAGAACGCATGCAGCATTCGGCTCTCATTCTGAGGTTCATCTGGCTG AAGTTTGAACTGTGCT
45	*P. radiata Phenylalanine ammonia-lyase*	ATCATCACCAGTGCCACCTAAGAACGCGTTTGTATTGAGATACCATCTATTTTTTCGGATGCAATTACTAGTTAATAATTT ATAACATTATTAGGGGTGGGGTCCAGAAAAATGAAAAAAGAAAAAGAAAATTGAAATTTTAAAACTAATTTTCAAAAT ATGAGGAAAAAAGCGAGACCACGAAAAAATCATTGAAAAAGACCTTGCAAAATTCAGGACTTGCTCTCACCAACCTCG CCAGGACTTTGACCGTGCTCATGCTTGTGTCATGCTTGCATATCTATACGTGTCACATCGACCGTCCGATCTATCATGAA AAGAACGGTCATGATGAAATCTCAACTAAACCCACTGCGTTAAATTTTCGAACAGTGAGAAAGTAATCGTATAAATACC CCTAAGCTCTTAGACCGAGAACGCATGCAGCATTCGGCTCTCATTCTGAGGTTCATCTGGCTGAAGTTTGAACTGTGCTC
46	*P. radiata Laccase*	AAAGATGCTACAATTTGATTTCTTTTTAGTTAAATTTAATCAGAAATATAGAAAAAGGTTAGGAAGATGTTTGCAGTCGT AAATATGAGCGCAATGGCCTTTAGTCCACGCGTAGTGGCACATCTTACACGGATACTTGGTTTTCAGCCCCACACAACTG CAAGGGTTGCTTCGAAGGTAACTCTTACGTTGGTTTGAGTGCCCAAAACATATTAGCTTTTTATTTTGTGTCACTGTCGAC ATCGTTGGCCCTAATTTTATCGTATGATCAGGCCCTGATCTCTCTCGCCACCATTTCCTTATAAGGCGCCAGCAGACAAG CACAGCTCTGGAAGGAACATGGGTGAGTGACATTAAAGCAACGCGATGACCTCATACCAGCTTCAACAGCTTACACCAT AAGACACGCTTTCCCATGGACATCCTCCTACGTATCACTCACTTGCCTATATATTCATGCAACTCCGTCACAGTTTTATAA TAATTCAGGTGCCTTTTATATCAGTAGTATCAACGGATACACCCAGGGTGATTGT
47	*P. radiata Cellulose Synthase*	AAATTCATGTTTGTCATAGGTTATGGTATTTTGCACACATGAAACAAATTTTACAATTGACTTTGATTAAGATATTAAATC TACAATAGGTTATCAACTCCACGTGATAATGAAGTAAAAAGACTGGATGGCTAAGTCAATAAAACAACCAAATAATCAA GCAATGATAGCTTCTATCAAATAAGGATGGTTCAGCTAGATCCAGGCGAAATATGATTCAGCCAGATACGAAAAGGCGA GCGGTTGAAATGTTTGAATGTTTGCGGGGTCCCTGGTTGCTTCGGAGGTTATTCTACGTAATTTATTCGTTATACCTTGCC TTCTAAGCATCGCAAACTGTGATTTCTTAACAAACTCGATGCATGCGCCATAACCAACAAAACCATTTAGTTGAGTTTAC GGTCTTCACAATTCATGCTCAGTCACCTTCAACTATTATGACAGATTAGGTGCTACTTATTCTCTCGTTACCCTTTAGAGT GAACTTTAATCCAAATTGTCAGGTGATTTGGGCCCCCAGGCGATGGATCCAGCGACAGGGGAACGCAAGTTTGGTGGTT GTGGCAGTGCAGTTGGTATGCCCCAGAGAGTTTTAAGACTTCAGATTTGTGTTCAGTATCAGGAGCTGCTATGGAAAAA GCAACCATATAAAACTATTGCCATTCGCACAGGAACAGAAC
48	*P. radiata LIM*	CCTTTGGGAATGAACTTTGAGACCACCTCCAACCCGGATTCTGAAATCCATCCAGCAATTCCAAAGTTCCAAACCGAAAT AAACATCCCACCATACCATGGCATTCGGAAAAAAGCTAGGCTAAGCTGAAAATCACTGTCATAACCCAGTAAGACCATG CCACTAATAGCAAGAGAACCATACACCAACATGCAAAGCCATGCATGTCCAAACCAGCTAGGAAATCACACATGCAAA GGGTTACCTGCAAGTATTCCTGTTGAAGTTGCTTGATCCTACTTTCTTTTCCTTGAGCCTTGCTTGCCTTCCTTTCCTTTGC TTGATTTTCCTTTCCTTGCTCCAAACTAGAGTGCTCTAAGAAAACTCTAAGTGACCAAGAGAGTGAGAGAGAGAGAGAA TAATGAGAGTCCAAACATGAACTTGACAAAAGCCATGAACTGATCCTCAGAAGTCATTTTATGCACGAGGCTTCTATTTT

		CTTCATTTTCCATCATTTTCCTTCAATTTCCTCATCACATGCAACGTGCGACTTTTCACCCCGTTTTCCTCCTAATTTCTTTT ATTTTCATAAATAAATGTGCCAAAAATGCCTcTTGCCTTAGCCTTTGCCAGTTTCCTTAGCCAAAACACACATCCAATGAT GCCCACTAGGATATCTTTGCCCAACATTAAGCCTGGAATAAATGTCTCTTAATCGTGGTCTTATTTTGCTTTTATTAACTT TTATTACATGAACTTTTCACTAAAGCTATTACAAAGATATATTTATTATGGCAATTATGTTTGATTTTTGAAGAGCTAGTA ACTTTTAGTTTATTATGGCCTTTTCCGTAAACTTATTTTCTTGAAAATCTCTATAAATCCAATGAAAAATTTATAGAATAT ATGTTGTGTTTTCTTCACTACCTCTAATAAATTTTTTACTTAGTAATCTACAAAGCCATTTATTAAAAAATTCAAGTTAAT TAAAAATTAATATCATTTCAAAAGTCTTTTTAATATAGTCAAAGTTTATTAAATTCTATGATGTATATTTCTTTTAAATAA ATGAAGAATCCATTTTTTTACTTAAAACCATATATTTTTTATAACGTTGATAAATAGCATGCATTTATATAAACAAATATA TATTTTTATAACGTTAAGAGATTGTTAAAACTTTTAAATAATTAATATTTTATTTATTGTTTTGAAAATGTCATGATTTCC ACCTACCTCGCCCATCAAATCTTGCTGCAAACCAGGCTTACCCAACCCCACACCCACAATATATTTTTGGGATCTGGTGC CCCCACCTTTGATCACAGTGAACACCATAAAGACAAATTATAAAGGCAAGGGGACTTGGCACCCATGAGGCAACCGAA AGCAACAAATCATTTTTTTCCAAAGAGATGAGTGTATGCCAACGAAGAAACACGATGAACCCACGTGTCATTGGCCAAC TCCCACTTTCGACAAAAAGAAGGAAATTAGAATTAAAAAGGCGAATAAAAATTGAAAGGCCATTTAAAATAGAAGGAA GAATAGCCTATATGGTAGATTTAAATGCTTTTTTGAAATCCGGTTACTCGCAAGATTATCAATCGGGACTGTAGCCGAAG CTT
49	*P. radiata Expansin*	AAATTAGTCAAATCCAAAGCAGACAACTTGGGCTCTCACCTAAATTAACACATATACCCTACCAGCTTCCATAGTTTCCA ACTTCCTTTCAATAAATCTATTCAAAAGCATGAAAAGCATGACTAAGGTTCAATTCCCAAGTTATGGACACCCACCTGCT CTAGGCATATAGGAAATCACAATCCAACTAACGACCAACTACCCAAAACTTTGAAGAAAATGAGTAAAGACTCCCCCAG TGATATTATAATTATATGGTCTCTCTAGAACCCTTTATTGCCCCTTCCAGTGTTATATTTAGTTCCCCATTTATATATCCCT TGACTTATGAAACCATTTAGGTGCATTAACATAGTCCTTGACTAACAAAAAAATTATTTAGGTGCAGTAGATACGGAAA GTAACCAATGATGCTAAGAAACTGTGCACGTACTTTAATGGAGGTATTACTTTTATTATGGTTGGTTTGGATACATTCAT AATGGAAGCATGTGCTCTTCATCGTTAAAGTTGTGGTGGGGCATTCCCCATTTTCCACGAGAAACCGAATCCCGGCGTGG AGACGACGACGAAATCGATGGATATTCGGTGGAAAATTCACAGTAAAATTCCTGGAGAAAAAGGTTGCCGAGGTAGTT GAAATCCAAACCGCCGAAATGAGCTGGAAACCCGCCTTCTGTCAGTTAGTTGAGTCATGACTGCAGCTGTCTCAGGTCTT ACACTGTAAAGGCACCTTAATGAGGCATTCATTCTGGCAGTCTGGCTACGGAACTTAATAGTACTTGTTATTCCTGCCCC AATATCTATTTAATAGGCATCCCCCCTCACTACTTCTTGCCCACAATCCCTCCATAGTCCTGAGCTTGAGACCATTTTTCT GC
50	*P. radiata 4CL3*	AAATATAACATAATCTAACTATTGATGTACATTATTCGCCTATAACAAAATCTAAGTATTGATGTCACATTATTGGCATA TAACAAAATCTTTAGGATAACCCCTTAGTCAAGCTCTTGTACTTTCATGTTTATTAACCAATAAATCAAGCTGATATGGA ATAGCAGACGTACGTGGTAATAATAAATGGAGTGTAAGAGTTCGAACATTTTAATTCGGAGGGGCAGCTTATGTGGAAT ATCAGGCAATCATACAAGCTTGCTTTTGGGTAATAAAGACCCACATGTGGTAATAACAAGTGGATTTTAACAAACCAAC ATTTTGATAGGGAGGATAGGTGGCCTGGTAAGTTAGAATGTGCTAGTCATGCCTTTGAAAGAAGTTAGTTGTGGAAGTC AAACATGTTCCCCACACAACACACCTCACCACACAAAATGCTGGTAGGTCATGTGATTGATGGATGGGCATGTGTATCC TCCAAAAAAAAATGAATATACACACTAAATATTCTATTGACATAATATACAAAGAAGATTAGGTCTATGGAAGAAGGGAA

		GGCGAAGGGGAAGATTGGGTCGTGGGGAAGATTGGGTCGTGTCCTGCTAGCACGTTGAATACCTACACGCCATTTCACA TCTACCCATCAACGTCAAATAGAGCATCCAAATCAGGGCGTGGTGGTGTGAGGGGAGAGTGAGGAGAAGAAGTTGAAA AATTCTGGCTGAAAATCCACCTAACACACGCTCACCAGCCCCTCAACGAGGGGCACCAATTATGAATAATAATAGCTAG AACAGAGCAGCAGAAGCAGAGTTTATATCTATCCATTGTCGTCTGTAAATTACTCTGTGAGTGTTTAGTGTTTTCTTCTCT TATTGATTTCAGGGGACAAGTAGGTGGG
51	*P. radiata 4CL1 or 2*	AAACACCAATTTAATGGGATTTCAGATTTGTATCCCATGCTATTGACTAAGCCATTTTTCCTATTGTAATCTAACCAATTC CAATTTCCACCCTGGTGTGAACTGACTGACAAATGCGGCCCGAAAACAGCGAATGAAATGTCTGGGTGATCGGTCAAAC AAGCGGTGGGCGAGAGAACGCGGGTGTTGGCCTAGCCGGGATGGGGGTAGGTAGACGGCGTATTACCGGCGAGTTGTC CGAATGGAGTTTTCGGGGTAGGTAGTAACGTAGACGTCAATGGAAAAAGTCATAATCTCCGTCAAAAATCCAACCGCTC CTTCACATCGCAGAGTTGGTGGCCACGGGACCCTCCACCCACTCACTCAATCGATCGCCTGCCGTGGTTGCCCATTATTC AACCATACGCCACTTGACTCTTCACCAACAATTCCAGGCCGGCTTTCGAGACAATGTACTGCACAGGAAAATCCAATAT AAAAGGCCGGCCTCCGCTTCCTTCTCAGTAGCCCCCAGCTCATTCAATTCTTCCCACTGCAGGCTACATTTGTCAGACAC GTTTTCCGCCATTTTTCGCCTGTTTCTGCGGAGAATTTGATCAGGTT
52	*P. radiata 4CL1 or 2*	ATCTTATGGAGTTTTTAAATATATATATATATTTTTTGGGTTGAGTTTACTTAAAATTTGGAAAAGGTTGGTAAGAACTATAA ATTGATTGAGTTGTGAATGAGTGTTTTATGGATTTTTTAAGATGTTAAATTTATATATGTAGTTGTGAAGGAGTGTTTTAT GGATTTTTTAAGATGTTAAATGTGTATATGTAATTAAAATTTTATTTTGAATAACAAAAAATTATAATTGGATAAAAAAT GTTTTGTTAAATTTAGAGTAAAAATTTTAAAATCTAAAATAATTAAACACTATTATTTTTAAAAAATTTGTTGGTAAATTT TATCTTAAATTTAGTTAAAATTTAGAAAAAAAAATAATTTTAAATTATTAAACTTTTGAAGTCAAATATTCCAAATGTTTT CCAAAATATTAAATTCATTTGACATTCAAAATACAATTTAAATAACAAAACTTCATGAAATAGATTAACCAATTTGTATG AAAACCAAAAATCTCAAATAAAATTTAAATTACAAAATATTATTAACATTATGATTTCAAGAAAGAGAATAACCAGTTT CCAATAAAATAAAACCTCATGGCTGGTAATTAAGATCTCATTAATTAATTCTTATTTTTTAATTTTTTTACATAGAAAATA TCTTTATATTATATACGAGAAATATAGAATGTTCTAGTCCAAGGACTATTAATTTCCAAATAAGTTTCAAAATCATTACA TTAAAACTCATCATGTCATTTGTGGATTGGAAATTAGACAAAAGAGAATCCCAAATATTTCTCTCAATCTCCCAAAATAA ACCTAATTAATATAGTTCGAACTCCATATTTTTGGGAATTGAGAATTTTTCTACCCAATAATATATATTTTTTTTATACATTTT AGAGATTTTCCAGACATATTTGCTCTGGGATTTATTGGAATGAAGGTTTGAGTAATGAAGGTTTGAGTTATAAACTTTCA GTAATCCAAGTATCTTCGGTTTTTGAAGATACTAAATCCATTATATAATAAAAACACATTTTAAACACCAATTTAATGGG ATTTCAGATTTGTATCCCATGCTATTGGCTAAGCCATTTTTCTTATTGTAATCTAACCAATTCCAATTTCCGCCCTGGTGT GAACTGACTGACAAATGCGGCCCGAAAACAGCGAATGAAATGTCTGGGTGATCGGTCAAACAAGCGGTGGGCGAGAGA ACGCGGGTGTTGGCCTAGCCGGGATGGGGGTAGGTAGACGGCGTATTACCGGCGAGTTGTCCGAATGGAGTTTTCGGGG TAGGTAGTAACGTAGACGTCAATGGAAAAAGTCATAATCTCCGTCAAAAATCCAACCGCTCCTTCACATCGCAGAGTTG GTGGCCACGGGACCCTCCACCCACTCACTCAATCGATCGCCTGCCGTGGTTGCCCATTATTCAACCATACGCCACTTGAC TCTTCACCAACAATTCCAGGCCGGCTTTCGAGACAATGTACTGCACAGGAAAATCCAATATAAAAGGCCGGCCTCCGCT TCCTTCTCAGTAGCCCCCAGCTCATTCAGTTCTTCCCACTGCAGGCTACATTTGTCAGACACGTTTTCCGCCATTTTTCGC CTGTTTCTGCGGAGAATTTGATCAGGTT

53	*P. radiata* *Pectate Lyase*	AAACAGAGCAGATAACACTAAAAAGACCAACCCTGTTAGGAGGGGAGAAACAAAAAAGATCACACTAAAAAGACCAA CCCTCTTATCTAAACTTATTTCTCTTATCTCTACCCCTTCTATTTTGAACCTTTATCATTTTGATAGAAAATATATGTTAA TAACCATTAAACCTACATTGTCAAGCTAGTGTAACTTATATGTTAATAACCATTAAACCTACATTGTCAAGTTAGTGTAA CTCCTTTGGTGGGGGTGGTTGTCTTCCTCTTCAATCTCATGCTATGACACACTTGTTTTTAATAACATAGGCCGACAAGT TTGAGCCATTATCTATCTTGATTCCTCGAAATGATAAATAGATGTTGTCAGTGGACTTGAAAAAAACCAAGTAGGGAAC ACCACGTAATCTTTCCAATGGCATTAAAAGCTACTTTGAAATATGTAACACTTAGCAATCCTTCCAAGGCATTAAACCTA CTCTAACCTATGGAACACTTAGCATCCTTCCCACGGTTGATAATAAATGATTGATTCCTCAGAATAACAAATAAAAAAA AACTATAAAACTTACTCTAAAATATAAAATGAGTATGGAACACGTGGCAATCCTTCCCATGCTCGGCGGTAGCTACTCTC TCCAGAGATTTGAATAACACAGGCGCCGCAATTATGAGAGAGCAGTGGAGTTAAGACTTAGTAGCCATGGTTATTTTGA ACGCGTGGCAATTCTTCCAAAGGTTGGTAGTTACTCTATCCAGAGATTTGAATAACACAAATGCTGCAGTTATGAGAGA GTAGTAGAGTTAAGTCTTGTCAGCAATGATAGTTACGAACAACCGTAATTTCTGGCTATCTCTGTGTTTATTGGTCGTTTA CTTGCTACAGTGCTCTCACCCCACATGGTAACAGTGTTCGATGGCCATGATTTCTCCCCACCCCGCCAAACCTCTACGTTT TTATTCTTTTAATAACTCCTAATTTAATATATAAGAGGGGGCAAGGTGTTCATACAGATTCGTGCAAACGACCTGAGTTC AGCACAAGTTTAGTCATTCCATGCGAACTCGACTGGCTCACGAGATCCCTCGCTGCAGTTATAGATTGCAGGAATTAGCT TAGCAGCATTTCTATCTATGATCTTCTGCCACTTCTTCCCCTCTC
54	*P. radiata* *ACC Oxidase*	AAGGTTTGCTTGGACCAGCGACACAGGGAAAAACATGGCATGCGGGTTTGGATTAAGATGAGGCCCAATCTTAATTTGA TATGTTTGCCAAACCTTAGGTTGTTTATCTAATTTTTGATTGGATCTGATCTCTTGATGATTTAAGGGTTTTCCATGTTGAC ACGCAATTGTAGGTTCCTGGGCACTAAGGTCTACCATGTGGCGAATTTATCGAGAGTTGACAATTCTGGTACTGTTAGTG ATTTGTCACCACTCTACGGTCCCTGCAGATCTCAGATTTTTAATGGCTGCCTTTGATTATCTAAAGGCTAGCCCCTAATCG CGGCTATGAATGTATAAAGAATGTGTTCCAATGCATTAGAGTACTCAAAGACATGTTGAAGGAAAAGGACAAGTCAAG GGACATGAGTAATAACCAAAAAAGCACTTGGTCCTGACCATCTGTGTCTGATTCACACTGGGATTCACATGTTATTTAAG AAAAGTTGCATCAGTGCTGCAATCATCAAGCCATTCCTAATTTACCACCATGATTAGATTATTTTAATGCAAGAAAACGC CTATATAAGGAGAGCTGCAGGCCCCAAGGTAATGCAGTAATCAAACTTGAGGAGAGATTTGAGAGTGTTTGTGAAGGG
55	*P. radiata* *Dirigent*	AAACGCTTCATGCCCCAGAAGCCGCACTCGATGCTTTAGAATAAAATGGACCATTACCAGACTACGCGCCTCCAAAATA ACAAAAACGTGTATTAGTTAAACCCTACATAGCACTTAAAGCTTGTCTTACTATTATTTTACGTAATTCTGTCTTTTTGAC AGTGGATTGATTGGAACTTCCATTCTCGATACAGTTGTATGCGTTATGTGAACTGAACCAACCTCGGCCAAAATATGGGG AAGATTCACTTCAGAAAAGACAGGACAACCATCTCTGATTGTCGACATTAATATCGGAAAAAATTCAGTCAAATGATGT GGAAAGGTTCATCTACGGAAAATAAAATAGCTCTGAGATGACCCGTTACATTTAGTGCATAGCATCTTTGTCAACAAGA AGAAATTTCCAGTTGTAGGACTGGTCATCAATGGCCGTGCCTGCAACGCTTTTTCGCAACAGGAAACACGGACTAAAAA ACGCGGTCTATCTGTCATTTGACGGTACGTTTGGCACTGAGCCCGAAAAAATCCCATTGGTAGAATTTAGAAGAGGGAG CTTTCACTCGAAAATTCTGTACCACAAGCGGTGGCCTCACAATAACAAATTATTATACCCACATGGAAAATGTTAAATCG GACGGTCCGACGGTCGACCAAAGACAAAATTGATGAGAAAGTTTTGAGGGTGGGTGATAAAGTAAGCGCGTCTTTTCAC AGGCATCTGCATTATAAACCTGCAACTCCAACTTTCATCACAACAAATTTCATTTTCCCCTTCTCTGAGGC
56	*P. radiata*	AAACAACAAAAAATAACAATCTACCTAGAAATTATATTACCAAATTTCAATTAAAAAACCCATTTCTTAGATTATTAAAC

	CSL	TACACCATTATAATTTTCATAATAACTACTAATACACCATTATAAATTTCATAATACTATTCATCCCATTATAAATTTCAT AATAACTTCTAATACACCATTATAAATTTCATAATACTATTCATCCCAATATGTGCTACCATTTAGATATTTTTGAGCCAA AACCCAACCCGAACAAAAATTTGTAATCTCGAGATTAATCACAAAATTTGACTCGATTCATATGCAAATTGGAATAATT ACTCGTCATGGATGAGATCTTACCGTTGGTGTGATCATGATGACGGCCAACTTTGGCACGCTTCATATCACAAATTGCAA CAACTACTCTGCTTTTAATGGATGACCATTGATGACGACCAAGCTTGACACGATTCATATGAGAGAAAGAACTCAAACA ATCTACTGCAATGTGAAAAGCCATGGGCACCGCCAAGATATTTAATTGTCCAACGCGTAACAATTAGTTACCCCCAATG GGCATTGGACTTGCCTTTGTCTTGATGTCGAAAACAAGGGGGGATTTCCTCTCTTTAAGAAAAATAGAAAAAACAAAAC CCCTGCACAGCTGGGTTCTCCTTTCTTCAAGCCTGGTTTGGCTTCAACATAAAGAAACAAAACCCATTCCATGGTGTTGT CTTATTGTGGGTTTGCCTAATTCAATGTTATTAGTGGTTGAAACTTCATTACAGCAGGATGGGAGAGCCAACCTCAAGAG AGTGACTCTGTAACCATCAATCTTCCGCATTGCCCTGCTGCCATGGATGTACTGGCGAAAATAAAGGGTCAACTTTGCTT AAAGATGCAGTCAGCTAGAGTTTAACTCAAGGAGGCAACCGGCTTCTATGTAATACCTGTGGAATGAAAACGAATCCCA TGTACCGAATTAAGGGAAAACTGGGTGCAGAGATTTTGTTTGGTTTAGACTCTAGATATGGTATTACAGCTCCGATTGGG TGGTCGAAATACGTCAGAGCACCCCACATTGCGTAATTCTTCAGGTATCAGATGCCTGCCTAGTCTACATACATGAGTTG CAGTTTCTCTTCAGCAGTGGGGTTTGGCGGCTCTGACAGTACAGTTAGTAGAGACTATCTATTTTCCGTGTACACAACGC TTGCAATGCAGATCTGGGCGCTATTATAAAAGATCAAACAAGAGCTAGGCTTTCAGAATTGCCTGAAAGCTGCTGCCAA TTGCATAGATCTGCTCAAGGCACCAC
57	*P. radiata CSL*	CTGTATTCATCACTTTACACCCATGATTCCAAACCCTACACATTTACACTGATAACCAAGGGTTCAGGTTCTTTCCAATTC ATTTTAATCCAGGATGATAATAAATTTGAATAGCACAATAGCATATTCCAACTGaCATATCCCTACATTTGGGATCTCTTT CCACGTTATAAATGGCTTCAATTTAGGGATCCCTTTCCACATTATATAACTGGGTTCACAGTGGTTTGAAGATAGCTGTG GTTTGAAGATAGCTGTATATGTTATCAAAATGACAGCTCCCTTGCCAGGGACCATCGCTTGAATGATGAGATCCCGCCTG TAAGGCAACTTGCAGCATGATTATTTTACATCTGCTTGACCAATTATCTAACAATATACGCGGTGTCGTCGTTCGGTTAA ATAATAGTGAAACTTCCTCGTGTTGTCCCTGCAGTTACGTATGTCTTGTTCTTTTTTTTGTTTAATAaCATACAGCAGAGCA AGTGTTGGGTGAATAAATATTGGGAAGAAGCTGCAGCGTTCACGTTCATTCATTCACTCATCGTGAGCAGCAGTACATC AACAGTTCTTGAAGAACATTGATAGGTTGGCTATTTCAATCCTTTCATGGGGAATATTTAAGTCTGGATCCGAGC
58	*P. radiata CSL*	ATCTTATCACATTTTCTCAAGAGAAGGGTTGTGACCAACTTTAAATTTTTGGTCTCTTTGATGGTGGTAAATTGGGGCAAT GAGACTCAACATTGTTAGAACATTTACCTTTCTCATACTTTGGAGGATCTATTAAGACAAAAGCTCTCATGTATTTCCTTT ACATGCATGCACATTTATAGGGAATAGAATGGAGTAGCAAATTGACTTTCTAAGGAAGGCCTACTCTTGACTCGGGGGT TGTGGCAGGTAGTTGAAGACTAGGGAGCCGGTCACTACCAATTTTACCATCAACCATTTACAGACGAGATACAAAATGA TGATTATGTTTAATTTTTGAAACTTTCACTTATTAATTTTTGTGACGCATTCATAACATATTATGTTAGTATATATGTTCGT TCACAGGTTGTTGGCTTTGGTAACACTATACTAGTATTTCTTTGTGATTATTTTTTATGTAATGCAATATAGCCCTAAATG AATATTGTGAAAGTGATATTTTTCAGGAGCATCAAGACCATCTTCATTTGTAAATATGTGATAAAAGGGGGGTGTGATA AATTTTAGTATTTTGTTATTTTTAATAAAATAGGAAGTGAAGATTATGTAAATATTATTTTCTAAATAAAAGGATATGAG AGAATAGTTTAGGAAAAAGAATTGGGATAGAATTTCTATGTTTTTCAATTAAAATTAGGATAAGAATGGAGAATAAAG CTTCACGCTTTAAATCATTATGTAAAACGGAAAAAGCCTGCTTTTGTAAAAGATAAGGTCTGAGAAGACCTATCCCTTAT

		GTATGTATCCGTTATTATTATAAATAAAGAGGTAGCTAATCTCTCAAGGGAGAGAGAGGAGCGAGCGCTCTGGAAAAAG ATGGATGATGTCTTGTTAATATTGTTAATATGGATGCGCGTAGTTAATAGTTTATTTGGACTGTGTATTAAGCATTGAATG GTTAGCTGTATATGTTATCAAAATGACAGCTCCCTTGCCAGGGACCTTCGCTTGAATGATGAGATCCCGCCTGTAAGGCA ACTTGCAGCATGGTTATTTTACATTTGCTTGACCAATTATCTAACGATATACGTGGTGTCGTTATTGGGTTAAACAATAGT GAAACTTCCTCGTGTTGTCCCTGCAGTTACGTATGTCTTGTTCTTTTTTTTGTTTAATATCATACAGCAGAGCAAGTGTTG GGTGAATAAATATTGGGAAGAAGCTGCAGCGTTCACGTTCATTCATTCACCCATCGTGAGCAGCAGTAGATCAACAGTT CTTGAAGAACATTGATAGGTTGGCTATTTCAATCCTCTCATGGGGAATATTTAAGTCTGGATCCGAGC
59	*E. grandis* *Laccase*	ATCAATTCAAGTAAAAAATTTTAATCCTAACTTAGTCATAAACTTTTATGCAATATTCCAATATAATCCGTCAGTCAATA TTAATCGGAATTGTTGACGTAGCGATGCGCCACGTAGAATGACTAACGATGGCTAAACCGCTATAGTAGCGATTTCTGA CAAATATTAACTGAATGACTATATTTTCCTCATTATTCAGGTTATATTGTTTTGTTTTCATGCTATTTCCCCAATAGCAAAT TTGTTCACCTGCTCCTGGAAATTCCTTACGACGACTCACCACTTATTCTAACGAATCTGATGGGTGATTCTTGATATTATT TGACCATGACATAATAAATGTCAAGGGAAAAAGAGAAAAAAATAAGAAAAGCGAAGAAATCCACCGGTCATCATTAGG ACAGACACATTATACGCCGTCATAAGGGAAAATGAAATTTAACTAAACATCACTAACGTCAACCAAACTCGAAAACAA AACTTGAACTGCAGTAGCTAGATGTAGCTCTTGGTTCAGCCCCCAGAACCATCGCCTATCGGGTTGATGGTTGAAGATGT GATCTTGGTCCTAATCACCTAATCAACGAACCACCGTTTCTCATTCGCTCCCTCCGTATAAAAACCTCGAGGCTTGTCCTA TCTTGGAGCATCGCATCCAAGAAACACCATCTCATCCTGTCTCAGTCCCCATCATCACTTG
60	*E. grandis* *Tubulin alpha chain*	GTCGTTTTTATATTGTCTAGCCACATTAGCATGAAAAACAATGTTGTTTTGCATTTCCTTTGTCGGAAAATTGCCGCGTTG GCATTTTGGTTGGAATGACACTTAAATGATCCATTTTGTTTTGATTTTGACACTTAAGTATTACTTTCCAAAGTTTTGACA CTTAAGTGTCCATTCGCACTAAGTTTTGGCATTTGAGTGTTCCTCCGTATCAAGTTTTGACATTTGTAATGTACTTTTGCTC ATAATGCTAATGTGATAATGAGACTAAATTAAACATATATTAAAATTTCAGAATCTACATTAAATAATTTAAAAATTTAT GAATCATATTACATATTACGATAAAGTTCAAGAACTATATTAAAAAAATTAAATATTTATGGGTCACATTACATACGAGT GAAAATTTAAGGACTATTTATTTTGTTATTTCTTTTTCCATTAACAAAAATCTTCCCCACCTCATTTTAAATTCGAGAAAA GAAGAAAAGCAAAGAAAAATAATAGAGAGGAAGGGACCCAACTCGAGATTGGGCTCCATTGATGGAAACTCGCGATCT ACTCCATCTCGACTCGACAGCCCATCCTCTGAAGATAACATCATCGTCCGCACCGCATTGCACCCTACCTTCTGGGCTGA ATGACCACATTGCCCCTCCACCAAATCTATCCGTTGCCTCGAATGCCGGATGGCAAAGCAGCAATTCCCGCAAAAGTCC GAGCCCATTTCCCTCCGGCCAAATCGAGAAAGGACTCTTGATTTTTGAAAACTGGGCGGGCAACTAACCTTGGTTAGGC GCCTCCATCATTAACCCCACACCAAAGTTAACACCCCCGCTTTCGCTGGCACTTTCTAAATCGAACCGCGGTTAACGTAA CCGCGGTTAACCAACCAGATATTTTTCAATTTTTTCCAGTGGCGCTCTATATATCTTTAAACTTCCCCTCTGCATTTCCCAT CAGCTCTGCAAGTCCTCCTCCATCTTCTTCTTCTTCATCGTCATCTTCTCGGAAGGCGTCTTGATAAAC
61	*E. grandis* *Coniferyl Alcohol glycosyltransferase*	ATCAAAGTTAGTCGCACTTTTACATACCCAACTGTACCTCCAAAGTGCACCATTGAACTTGTGACAACGTTTAGATTTAG GTAATTATTCAGAAAACGAAAGCGACCACAGGTTTATGAATTGTCACGCATGACGTCATTAATTAAGCGACAAGACGTG CGCCAAGGCCATGCATTCCTCTGCGGCTATCCTTCTTCCTGGCAACAGTTCAATTCCTCAGACGGTCTGGTCAAACCCGA AGCTCGACTAGGCCTTTCTCAACCAAACCCTCCAAGAAAGCCTAAGGACAGCATGCCCTCGCGCGGATCAACACGACCG ACGAGCATCGAACTTGCGTAACTTACCCCACCAAACGGTCCCCTTCGAGGTCAAACCCCACGCGAACGACCGATGAATC

		GAACATCTAATCTCGCCTCTCCTCTCCTCCACTGCTATATATTTCAGCTACTCTAACACACTCTCATCACCACCAACTTCA AACTCTCTCTCTCCCTCCCTCCCTCCCTCTCTCGCTCACACATACACACAT
62	*E. grandis CONSTANS-like B-box Zinc finger protein*	CTGGAGTTCACATTGAGCTGGTGCCGATCGATCCGTTTCTTACATTTTTTCATCCCGGTCCGTCTCCATTCTCTGCCTCCGT CGGCATCTTGGGCGACGAGAGGAGGAGGAGATACGCGGTAGCTGACGAGTTCGAGGCGCAACTTTTCTTCGATTAACTT TTAACTCGACACCGATCATGCTTTAAGCACTTACCCTTTTCGAGAAACAGGAGATGGACATGGAGTTCGACGTAAAGAT CCCGTCTTTGATTACAGAAAAAGCATGCTCAGAGGAGGAGGAATGATATTTCCTGTTTCCATGGTGGTGATAAAAGCTTT GATTTTTCCTTTTCAATGACGTAGCTCGAGTGGCCGATAATCGACAAGGAGGTCCAACTATTAGCACCAGAATGGAAAA GAAGAGGGAGATAGATAGCGACTACCACAAGCTACATTACAAGGATTAATATAAGCAAAATTACTGCAATACGATATT GACCCGATTGGCTTTGGATGATAAAAAAACAATTCTATATTCAATCACACGTCTTCGTCCCCGGGAAAGCAATGATCCA AATCATGTCAAGGAGCTATACTCCTAAGCCCACGTTAGCCCACACTCTTCTCGAAAGACATATCAAATCAATACACTCAC TCTCTCTATTAATATTCAATTTCTGCATAATTTCTTCTGTCACTGCCCAAGACGTTCTGTAGCACTAAGGGGTG
63	*P. radiata Alpha Expansin*	AAATTCACATTCTTTTCTTCGCACGAAGAAAGGTTAAAGATACAACTCGGATTGTATTAAAGGAAAGAGATTGGAACA AACAGAATCTGGAATATAAGAATACACCAGATCGCGGGCACGGCCACAGTTTAACGGCCAGCCGAAAGGCCGGTCCGT TGGGTCTGCCGGTGACTTGGTCGTGTGAGGGAATCTCTGGAGTCCGGATCCGGTCTTGCCTTGAGACCTACCACAACCAC AGCAGTTAATGCAGTTTACATCCTATTAATATAAATACCAAATCGCCATTCCAAATTATTATCACAACAACAAATCTGAT TTGATTTCGATGCAGTGAAGCTCTTCATTTTGCAGTGACAGTGACGTT
64	*P. radiata Tubulin Beta Chain*	GGACAAACGAGATTTTATTCTCATCCAGTTCCATCTATTCTCTGTCACTGTAACTTGTAGAGATTATATTAACGATGGGGT TATGATCGCTTCACGTTTCCAGATAGAATGGAGAGAACAACAGCAAGGAAATCGACAGGCCATAACTTAATGGGGTCAC TGTAAGGCCTTCCGGGGCGTAAACACGAAGCTTTGTACAGAGAGTCCACCCAAAAACAAGCATCATCACAGTGACAATA ATTGAAAAAGAAATGAAAAGCTCCACTGGGCTTCTCTTTCTGGAACCTTCTCTCCGAAGAAATCGACTTACAGAATTTAA AAAATTTAAAATGATGTTCTGTAGCAACCTAGGCCCTCCACTGTCACCATACCTGCCCCTCCATTGTCACATTCTATCTTC TCATCTTAAACACCACGCATCTCGCTTTTCCACTGCATGCAGAGATCGACGATATCTTTGCTTGATATCTAAGTCGAATTC TGACCGCAAACCTCCATCAGACTTGCGCACATCTTAATAGATGGCGCTTGTTTGTGCCCAAGGGGTTCTGGGTACTATTT GAGGACTGAAGGTGTTATGCTTCAGAGATTTGGAGGCCTAGGGTTCGATTCACAGCCGTTGAGATTTCGACAGAATTTG GATTTTTTTTCTCTGGCTGTTTGAGGAGAATGAGAGAGATATTGCACATCCAGGGCGGGCAGTGCGGGAACCAGATAGG AGCCAAGTTCTGGGAAGTGATATGTGACGAGCATGGGATTGATACCACGGGCTCGTACTGTGGGGACTCCGATCTGCAG CTGGAGAGGATCAATGTCTATTATAACGAGGCAAGCGGCGGCCGCTATGTGCCTCGGGCAGTGCTGATGGATCTCGAAC CCGGGACCATGGACAGCGTTCGATCAGGTCCCTATGGTCAGATCTTCAGGCCAGATAACTTCGTCTTTGGGCAGACAGG CGCCGGGAACAACTGGGCCAAAGGGCATTATACTGAGGGGGCAGAACTCATTGACTCCGTTCTTGATGTCGTGCGTAAG GAGGCCGAGAGCTGCGATTGTCTTCAGGGATTTCAAGTATGTCATTCCCTGGGAGGAGGAACAGGATCGGGAATGGGGA CTCTCTTGATTTCCAAAATAAGGGAGGAGTACCCAGACAGAATGATGTTGACTTTCTCTGTTTTTCCATCACCTAAGGTA TCGGACACAGTGGTGGAACCTTATAATGCAACTCTTTCTGTACATCAATTGGTGGAGAATGCAGATGAATGCATGGTTCT TGACAATGAAGCACTTTACGACATTTGCTT
65	*P. radiata*	GTATCATTATTTCAGTCATTATCGATAATGATAAGCCTCAAATATGAATCAATAGTCTCTTAGTCATTTAATTTATGGTTT

	Putative Beta 1,3-Glucanase	TCAGTGTCGATGTGCTCTCCTGCCAGGGCTCCACCAATCTCCTTTAGGTTCAGTGTACATCGTCTGAAAATAAGTTGACA AGGCCAGGTCAATGCAGAAGCCTCCTGGCTTGGGGACCCTAAGTGTGAAATCAATATATTTTCCTCGAGTTCTTGACCTG TTAGCAACTTCGACACTGCAACTTGTCCTAATCTTTGCTGTGTATTATGTATTTGTTCCAAGTATTGGAGTGTAGCACAG TGGATGGTAGAGAGGAGGATCTAGATCAGTCACTTTTACATAGAATGGAGATGATAGTAAAAGCAACTACAATTACGAT CTTGCTACCAGTCATCCTATGTTGCATCCCATGTGGAGAAAGTGGAAGCGGAGGCAGGAGTTTGGCGCAGCGTTTACCA GCCCTAGGCGTTGACTATGGACAAACTGCAGACAATCTTCCTCCACCATCTGCAGTAGCAAAGCTGGTTCAGAGTACAA GTATTTCAAAGTTGAGACTATATGGAGCAGATCCTGCAATTCTTCAAGCATTTGCTAACACAGGAATTGGGTTAGTTGTA GGCATTGGTAACGATCAAATCCCATCTCTGAACCAGCTGGCTGTTGCACAGAATTGGATTAAGAACAATATCGTTCCTTT TGTTCCTGCCACTGATATCATTGGAATCTCGGTGGGGAACGAGGTTCTGTTCAGTGGGGATGGAGTCTGATTTCCCAGC TCCTCCCTGCATTGCAGAACCTACACACTGCCCTTGTTGAGGTTTCACTTGACCAGCAAATTAAGGTCTCCACACCTCATT CTCTGGCCATACTTTCTACATCTGTCCCCCCATCTGCTGGCCGTTTCAATGAAAGTTTTGACATGAAATCCCTGCTTGACT TCTTGCAGAAGATAGGGGCCCCATTAATGATCAACCCATACCCCTACTTTGCTTACAAGAGTAATCCCACCGATCAAACC CTGGCTTATGCACTCTTCGAGCCCAACCCGGGCTTCTATGACACAAACAGTGGGCTCACCTATACCAACATGTTTGATGC TCAGCTTGATGCAGTGTACTCAGCCATGAAATATCTGGGTTACCCTGGTGTTGATATAGTGGTGGCTGAAACAGGATGGC CAGCTGTGGGGGATCCTACAGAGACAGGGGTGAGCTTACAGAATGCAATTGCTTACAATGGCAACCTGATCAAGCATGT GACGTCCATGACGGGGACCCCATTGAGGCCAAATAGGTACATTCAAACCTATATTTTTGCCCTCTTTAATGAGGATCTGA AGCCAGGACCAACTTCGGAGCGCAATTATGGGCTGTTTAAAGTTGATATGACAATGGCTTATGATGTGGGTTTGTTGCAA TCGCCGAGTGCAGCTCCATCTCCTCCTGCTCCACGCACTGGGGGGCCTGTGACAACTCCTCCTACAGGTAAAGTTTGGTG CATTGCCAAGCCGGGCGCCGAAGAGCAAACTTTGGAGGCAAATTTGAACTATGTTTGTGGACAGGGCATTGACTGTAGG CCTATTCAACCAGGAGGTCCTTGCTATTCACCAAATACAGTGGCAGGCCATGCTGCTTATGCCATGAACGCATACTATCA GACTGCGGGTCGGAACAATTGGAATTGTGATTTTGCGCAGACGGGAACTCTTACCTCCACAGATCCAAGCTACGGGGCC TGCGTGTACCCGACCGTCTAAGATATGAATCAATCAATCAATCCTAGTGTTTTCTATTCCACTTGTTGTTCGGTATATATT TTCCAACTTGTCTTTTCATATGAGTGAGATGTATGAGGTACCTATTTTCAAAGTTGTGATAGCATATACATCATAAGATGT AATGTGTATGTTTGGGTTTTATTCCCCCTTTAATGTCGTTACTCTGACCATATAAAAAAATTCACAGAATTTGTGAATGGT AGTATTATTTTTTATTTATGTATTAAGGAAATTTAAGTGGTGTTAAAAAAAGGAAAAAAAA
66	*P. radiata Cinnamoyl-COA Reductase*	AAAAAAAATTATGATCTGTAAATAAATATAATTCCATATATAATAGATATATATAAATTTTACAACCCACAGATAAATAT TAACTTTCCGCCAAAATAATTTCCATAAATGAAATAAATGACCCAATATTACGATTTTACACCAAAATGATTTCCATATG TATATATAAAGCCTGTGAGTCCAAACGAAGCATATGAATCTGAATCGCAGAGGGAGGCTGGCCAACCACCATTAGCTAT TCAATGAAGTTGGTAGCCACCCAAACAAGTCAATTCAAGAGTCAATCAAACCAAACTATGATTAAAACTACCAACCGCA CTTTCTGAGCAACCCACTTTCCCTCCCTCGCTTTACTTTTGGAGTCGTGGGGGATTTTTCCAGTGTCTCAATTTCTATAAA TTTGGCCTCACATTTCCTACCAACTCATTGTTAACGGGAGTCCTCTTGTCAGGCTCCGCTGCTTCTTGTGATCACACGATA CCTAGTGATCCATAGATAACTAAAATGCTGTGAGCAGTCTGAATTCTTGCTTTCTTTCCCC
67	*P. radiata Dirigent*	GTCTGTATTATTATATTCTGGGTCACTACTCAACCCCACGGTAGTGGCGTGACTTGCGTCGGCGTGTTACAGAATCCATA ATCAGAAAACGAACGGAAGCTGCAAAGGTGTACGTCCAACGGTTGCGGTGAAAAGCCATTGGTTACGTCCAGCGGTGG

		AATTCTGTAATACTGAAAGGATTTGGTTACAGATGGCTCGACCAAAGACAAAATAGTAATCAAATATTCAACCGAAAGG GAGAAAGTTGCTTATGGGCATCACGTTATAAAAGTGGAACTCGACTTTCATTACCACACATTTCTCATTTCTTTCTCTGTA CTGAGCCATTCGTTCTCCTTTCTTTCAGAGA
68	*P. radiata* *Caffeic Acid OMT*	CTGGCAACTGGCTATTCCTCATTCGTCAGTGGGAATGGGGTGGGCAGACGATCTTCTAGAGCCTGTGTGGTGTGGGGCCC TTCGACTTTTCAATGGCCCGTTGGTCACCAGCTTGGACTAGTTTTGCTGTTTCCATGGTGACGGTTCGTGCTCTATAAAAT AATTTAACCGAGTGGGTATTTTGCATGGTGGCCGGATTTCCAACAATCTCAGGTATTAGCG
69	*P. radiata* *High Mobility* *Group* *Transcription* *Factor*	ATCTAACCCACGATCTATAATAATAGTCAAGGACCCTAAATAGAAATATGGCCACCACCCTACCACGAGAGCTTATCCT AATACAACCACGAAAGCCCCTCCACTCGTGGAGGTTATAGATTTCCCCCGTGTAAACATATAAAAGGAACTTTTCTCTTT GGTGACCGGCAACAACCGGATACTCACCCGGTATCGCCGAAGAAGCTTGTTGCGAGGTTCGCATTGAAAACCCTCCTCT CTTCACATTCTTTGCCGGTCATCCATCTTGCTCATTTCTACTTCCGCCTCCTCTTCTCTTCCCTCGTCTAGTGTTTTCTTTGC GTTGTGTAGTGTAATGTTTGCTGTTGCTTCATATCAATAGTGGTGGAATTTTCCTTCACTGCGAGCAGATTTTCTAAGGAG A
70	*E. grandis* *Tubulin alpha* *chain*	GTCGTTTTTATATTGTCTAGCCACATTAGCATGAAAAACAATGTTGTTTTGCATTTCCTTTGTCGGAAAATTGCCGCGTTG GCATTTTGGTTGGAATGACACTTAAATGATCCATTTTGTTTTGATTTTGACACTTAAGTATTACTTTCCAAAGTTTTGACA CTTAAGTGTCCATTCGCACTAAGTTTTGGCATTTGAGTGTTCCTCCGTATCAAGTTTTGACATTTGTAATGTACTTTTGCTC ATAATGCTAATGTGATAATGAGACTAAATTAAACATATATTAAAATTTCAGAATCTACATTAAATAATTTAAAAATTTAT GAATCATATTACATATTACGATAAAGTTCAAGAACTATATTAAAAAAATTAAATATTTATGGGTCACATTACATACGAGT GAAAATTTAAGGACTATTTATTTTGTTATTTCTTTTTCCATTAACAAAAATCTTCCCCACCTCATTTTAAATTCGAGAAAA GAAGAAAAGCAAAGAAAAATAATAGAGAGGAAGGGACCCAACTCGAGATTGGGCTCCATTGATGGAAACTCGCGATCT ACTCCATCTCGACTCGACAGCCCATCCTCTGAAGATAACATCATCGTCCGCACCGCATTGCACCCTACCTTCTGGGCTGA ATGACCACATTGCCCCTCCACCAAATCTATCCGTTGCCTCGAATGCCGGATGGCAAAGCAGCAATTCCCGCAAAAGTCC GAGCCCATTTCCCTCCGGCCAAATCGAGAAAGGACTCTTGATTTTTGAAAACTGGGCGGGCAACTAACCTTGGTTAGGC GCCTCCATCATTAACCCCACACCAAAGTTAACACCCCCGCTTTCGCTGGCACTTTCTAAATCGAACCGCGGTTAACGTAA CCGCGGTTAACCAACCAGATATTTTTCAATTTTTTCCAGTGGCGCTCTATATATCTTTAAACTTCCCCTCTGCATTTCCCAT CAGCTCTGCAAGTCCTCCTCCATCTTCTTCTTCTTCATCGTCATCTTCTCGGAAGGCGTCTTGATAAAC
71	*P. radiata* *WD-40 Repeat* *Protein*	AAATAGGCTAAATTAGAGAAATACTATGGGTTGTCAAAACCTAGAATACGATAATTTGACCGAAATATTTAGATAATGT AACATAACATGACATGACATTACAACATCTCTTCCATAGAGAATCTCTCAATAAAATAAAATATTGCACAAACAAAACC AACTCAAAACTCAATTTATATTACACAATATAATAATAAACAATTTCAATTAAAAACATTTTACCTTTATTTATTAATAA ACCTCACACTAACACATTGTTAAAAAAGTAAAATAAAATAACAAACGCCATATAAACCCATAAAAATTTCCAAAACAAT ATTAATATCTTTATCATAGTTTTTAAGCTAAAGTTCGATGATCCTTTAACATTACTAGCCACAAGGATGCTTACTTCCTTG CAAAATAACAATGCAAAGACCCAACGCAGTGATATGTGATTTAACGGTAAGTATGGTTGGGTGAAACCAACAAGACTG CAGTTCAAATTCCATTGAGTATATGGCCTGCTATGATCTCAGCTTGGTGAAACCAACAAGACTGCAGTTCAAATCTAAAT CCCATTAATTATGTGACCTACTATAATCTGGGCTTAAGGAGTAGGTTGCTCGCTATGTTTTGGTGTTATAAAGTAGCCAT AAAGATTAAACCTCAAGCTCCCCTAAATTAATCCAAGAAATTACCGATTCATTATAATTAAAAAAAATGCAAATACCCA

		CCTTAAAGAAAAACAATGTAAAGAGCAATGAAATCAATTTAATTGTCTTCTTTTAACACCAATAAAAATTTATAAAAAC CTCATAATTAAAAACAAAGCGTTAGACTTTTGGAATAACCTTCCTTAATTGCTTCTCTAATTTATGATTTCTAAGTCATAC CACGATCGGTCGTTTTAGCAAAAGCCTGAAAGGCAAGTAGAAGATAAACGTATGCTTGGAAATAAATATATGTCATTTT TCATTTTATATCCTTCGAATCCGTCATTCGTCTGAATGATCAGACAAACCCTCCCAGATCCTGCTCTGTTCTGAAGCATAA ACCT